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 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2           "Ask CAS" for self-help around the clock
 NEWS  3  FEB 27  New STN AnaVist pricing effective March 1, 2006
 NEWS  4  MAY 10  CA/CAplus enhanced with 1900-1906 U.S. patent records
 NEWS  5  MAY 11  KOREAPAT updates resume
 NEWS  6  MAY 19  Derwent World Patents Index to be reloaded and enhanced
 NEWS  7  MAY 30  IPC 8 Rolled-up Core codes added to CA/CAplus and
                  USPATFULL/USPAT2
 NEWS  8  MAY 30  The F-Term thesaurus is now available in CA/CAplus
 NEWS  9  JUN 02  The first reclassification of IPC codes now complete in
                  INPADOC
 NEWS 10  JUN 26  TULSA/TULSA2 reloaded and enhanced with new search and
                  and display fields
 NEWS 11  JUN 28  Price changes in full-text patent databases EPFULL and PCTFULL
 NEWS 12  JUl 11  CHEMSAFE reloaded and enhanced
 NEWS 13  JUl 14  FSTA enhanced with Japanese patents
 NEWS 14  JUl 19  Coverage of Research Disclosure reinstated in DWPI
 NEWS 15  AUG 09  INSPEC enhanced with 1898-1968 archive
 NEWS 16  AUG 28  ADISCTI Reloaded and Enhanced
 NEWS 17  AUG 30  CA(SM)/CAplus(SM) Austrian patent law changes
 NEWS 18  SEP 11  CA/CAplus enhanced with more pre-1907 records

 NEWS EXPRESS  JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 JUNE 2006.

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 NEWS IPC8     For general information regarding STN implementation of IPC 8
 NEWS X25      X.25 communication option no longer available

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FILE 'HOME' ENTERED AT 13:31:23 ON 20 SEP 2006

=> file reg
COST IN U.S. DOLLARS                              SINCE FILE     TOTAL
                                                       ENTRY   SESSION
FULL ESTIMATED COST                                     0.21      0.21
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DICTIONARY FILE UPDATES:   19 SEP 2006  HIGHEST RN 907944-91-6

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experimental property data in the original document.  For information
on property searching in REGISTRY, refer to:

http://www.cas.org/ONLINE/UG/regprops.html

=> E "SERPINA"/CN 25
E1            1      SERPIN-LIKE PROTEIN (LUMPY SKIN DISEASE VIRUS STRAIN
NEETHLING_WARMBATHS OPEN READING FRAME LD149)/CN
E2            1      SERPIN:ELONGATION FACTOR, GTP-BINDING:ATP/GTP-BINDING SITE MOTIF
A (P-LOOP):TYPE 2 KH DOMAIN:SMALL GTP-BINDING PROTEIN DOMAIN... (BRUCELLA
MELITENSIS BIOVAR ABORTUS STRAIN 2308 GENE ERA)/CN
E3            0 --> SERPINA/CN
E4            1      SERPINA1 (HUMAN)/CN
E5            1      SERPINA1 PROTEIN (DANIO RERIO CLONE IMAGE:6997096 GENE
SERPINA1)/CN
E6            1      SERPINA1 PROTEIN (DANIO RERIO CLONE IMAGE:7049407 GENE
SERPINA1)/CN
E7            1      SERPINA10 PROTEIN (HUMAN CLONE DNA64890 GENE UNQ707)/CN
E8            1      SERPINA10 PROTEIN (HUMAN CLONE MGC:22287 IMAGE:4710734)/CN
E9            1      SERPINA10 PROTEIN (MOUSE STRAIN FVB/N CLONE MGC:25863
IMAGE:4196269)/CN
E10           1      SERPINA1A PROTEIN (MOUSE STRAIN FVB/N CLONE IMAGE:4160332 GENE
SERPINA1A)/CN
E11           1      SERPINA1A PROTEIN (MOUSE STRAIN FVB/N CLONE IMAGE:4209679 GENE
SERPINA1A)/CN
E12           1      SERPINA1A PROTEIN (MOUSE STRAIN FVB/N CLONE IMAGE:4210562 GENE
SERPINA1A)/CN
E13           1      SERPINA1A PROTEIN (MOUSE STRAIN FVB/N CLONE MGC:13995
IMAGE:4193647)/CN
E14           1      SERPINA1A PROTEIN (MOUSE STRAIN FVB/N CLONE MGC:13996
IMAGE:4211464)/CN
E15           1      SERPINA1A PROTEIN (MOUSE STRAIN FVB/N CLONE MGC:28457
IMAGE:4160260)/CN
E16           1      SERPINA1A PROTEIN (MOUSE STRAIN FVB/N CLONE MGC:29136
IMAGE:5052898)/CN
E17           1      SERPINA1A PROTEIN (MOUSE STRAIN FVB/N CLONE MGC:30253
IMAGE:5123976)/CN
E18           1      SERPINA1A PROTEIN (MOUSE STRAIN FVB/N CLONE MGC:36173
IMAGE:5099206)/CN
E19           1      SERPINA1B PROTEIN (MOUSE STRAIN FVB/N CLONE MGC:18593
IMAGE:4194027)/CN
```

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E20          1     SERPINA1B PROTEIN (MOUSE STRAIN FVB/N CLONE MGC:29137
IMAGE:5053771)/CN
E21          1     SERPINA1D PROTEIN (MOUSE STRAIN FVB/N CLONE MGC:30252
IMAGE:5125194)/CN
E22          1     SERPINA1D-PROV PROTEIN (XENOPUS LAEVIS CLONE MGC:64429
IMAGE:6879954)/CN
E23          1     SERPINA3G PROTEIN (MOUSE STRAIN MIX FVB/N, C57BL/6J CLONE
IMAGE:5250914 GENE SERPINA3G)/CN
E24          1     SERPINA3K-PROV PROTEIN (XENOPUS TROPICALIS CLONE IMAGE:6999066
GENE SERPINA3K-PROV)/CN
E25          1     SERPINASE/CN

=> S E23
L1           1 "SERPINA3G PROTEIN (MOUSE STRAIN MIX FVB/N, C57BL/6J CLONE
IMAGE:5250914 GENE SERPINA3G)"/CN

=> DIS L1 1 SQIDE
THE ESTIMATED COST FOR THIS REQUEST IS 6.36 U.S. DOLLARS
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   592464-45-4  REGISTRY
CN   Serpina3g protein (mouse strain mix FVB/N, C57BL/6J clone
     IMAGE:5250914 gene Serpina3g) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   GenBank AAH57144
CN   GenBank AAH57144 (Translated from: GenBank BC057144)
FS   PROTEIN SEQUENCE
SQL  350

SEQ      1 LKFNLTETPE PDIHQGFRYL LDLLSQPGNQ VQISTGSALF IEKHLQILAE
        51 FKEKARALYQ AEAFTADFQQ PLKATKLIND YVSNHTQGKI KELISGLKES
       101 TLMVLVNYIY FKGKWKNPFD PNDTFKSEFY LDEKRSVIVS MMKTGYLTTP
       151 YFRDEELSCT VVELKYTGNA SAMFILPDQG RMQQVEASLQ PETLRKWKNS
       201 LKPRMIHELR LPKFSISTDY SLEHILPELG IREVFSTQAD LSAITGTKDL
       251 RVSQVVHKAV LDVAETGTEA AAATGMAGVG CCAVFDFLEI FFNRPFLMII
       301 SDTKAHIALF MAKVTNPERS MNFPNGEGAS SQRLESKRLC FGDPLCLIGQ
MF   Unspecified
CI   MAN
SR   GenBank
LC   STN Files:   CA, CAPLUS
DT.CA  CAplus document type:  Journal
RL.NP  Roles from non-patents:  BIOL (Biological study); PRP (Properties)
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> file caplus
COST IN U.S. DOLLARS                                 SINCE FILE     TOTAL
                                                          ENTRY   SESSION
FULL ESTIMATED COST                                       12.00     12.21

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FILE LAST UPDATED: 19 Sep 2006  (20060919/ED)

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=> s l1
L2             1 L1

=> d ibib

L2   ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:         2004:979659  CAPLUS
DOCUMENT NUMBER:          142:18207
TITLE:                    The status, quality, and expansion of the NIH
                          full-length cDNA project: The mammalian gene
                          collection (MGC)
AUTHOR(S):                Gerhard, Daniela S.; Wagner, Lukas; Feingold, Elise
                          A.; Shenmen, Carolyn M.; Grouse, Lynette H.; Schuler,
                          Greg; Klein, Steven L.; Old, Susan; Rasooly, Rebekah;
                          Good, Peter; Guyer, Mark; Peck, Allicon M.; Derge,
                          Jeffery G.; Lipman, David; Collins, Francis S.
CORPORATE SOURCE:         The MGC Project Team, NIH, USA
SOURCE:                   Genome Research (2004), 14(10b), 2121-2127
                          CODEN: GEREFS; ISSN: 1088-9051
PUBLISHER:                Cold Spring Harbor Laboratory Press
DOCUMENT TYPE:            Journal
LANGUAGE:                 English

=> s 2A2 or AI119734 or alpha-1 antiproteinase or alpha-1 antiproteinase or
MGC107057or Serine protease inhibitor 2A or Serine protease inhibitor A3G or Serpin
2A or Serpin A3G or Spi2/eb.1 or Spi2-1 or Spi2A
'EB.1' IS NOT A VALID FIELD CODE
           859 2A2
             0 AI119734
       1633624 ALPHA
          2486 ALPHAS
       1633730 ALPHA
                 (ALPHA OR ALPHAS)
       8829298 1
           460 ANTIPROTEINASE
            88 ANTIPROTEINASES
           504 ANTIPROTEINASE
                 (ANTIPROTEINASE OR ANTIPROTEINASES)
           155 ALPHA-1 ANTIPROTEINASE
                 (ALPHA(W)1(W)ANTIPROTEINASE)
       1633624 ALPHA
          2486 ALPHAS
       1633730 ALPHA
                 (ALPHA OR ALPHAS)
       8829298 1
           460 ANTIPROTEINASE
            88 ANTIPROTEINASES
           504 ANTIPROTEINASE
                 (ANTIPROTEINASE OR ANTIPROTEINASES)
           155 ALPHA-1 ANTIPROTEINASE
                 (ALPHA(W)1(W)ANTIPROTEINASE)
             0 MGC107057OR
        109032 SERINE
```

```
          1771 SERINES
        109700 SERINE
                 (SERINE OR SERINES)
         95580 PROTEASE
         35069 PROTEASES
        111486 PROTEASE
                 (PROTEASE OR PROTEASES)
        518101 INHIBITOR
        525022 INHIBITORS
        817793 INHIBITOR
                 (INHIBITOR OR INHIBITORS)
         37227 2A
             0 MGC107057OR SERINE PROTEASE INHIBITOR 2A
                 (MGC107057OR(W)SERINE(W)PROTEASE(W)INHIBITOR(W)2A)
        109032 SERINE
          1771 SERINES
        109700 SERINE
                 (SERINE OR SERINES)
         95580 PROTEASE
         35069 PROTEASES
        111486 PROTEASE
                 (PROTEASE OR PROTEASES)
        518101 INHIBITOR
        525022 INHIBITORS
        817793 INHIBITOR
                 (INHIBITOR OR INHIBITORS)
            33 A3G
             0 SERINE PROTEASE INHIBITOR A3G
                 (SERINE(W)PROTEASE(W)INHIBITOR(W)A3G)
          2144 SERPIN
           940 SERPINS
          2348 SERPIN
                 (SERPIN OR SERPINS)
         37227 2A
             7 SERPIN 2A
                 (SERPIN(W)2A)
          2144 SERPIN
           940 SERPINS
          2348 SERPIN
                 (SERPIN OR SERPINS)
            33 A3G
             1 SERPIN A3G
                 (SERPIN(W)A3G)
             0 SPI2/EB.1
           123 SPI2
       8829298 1
            12 SPI2-1
                 (SPI2(W)1)
             6 SPI2A
L3        1038 2A2 OR AI119734 OR ALPHA-1 ANTIPROTEINASE OR ALPHA-1 ANTIPROTEIN
               ASE OR MGC107057OR SERINE PROTEASE INHIBITOR 2A OR SERINE PROTEA
               SE INHIBITOR A3G OR SERPIN 2A OR SERPIN A3G OR SPI2/EB.1 OR SPI2-
               1 OR SPI2A

=> s l3 not py>2002
       4356482 PY>2002
L4         866 L3 NOT PY>2002

=> s inhibit?
L5     1863727 INHIBIT?

=> s l5 and l4
L6         147 L5 AND L4

=> s contact? or administ?
```

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        640187 CONTACT?
        641848 ADMINIST?
L7     1277182 CONTACT? OR ADMINIST?

=> s l7 and l6
L8           9 L7 AND L6

=> d ibib 1-9

L8   ANSWER 1 OF 9  CAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:         2002:937303  CAPLUS
DOCUMENT NUMBER:          138:20443
TITLE:                    Endocrine disruptor screening using DNA chips of
                          endocrine disruptor-responsive genes
INVENTOR(S):              Kondo, Akihiro; Takeda, Takeshi; Mizutani, Shigetoshi;
                          Tsujimoto, Yoshimasa; Takashima, Ryokichi; Enoki,
                          Yuki; Kato, Ikunoshin
PATENT ASSIGNEE(S):       Takara Bio Inc., Japan
SOURCE:                   Jpn. Kokai Tokkyo Koho, 386 pp.
                          CODEN: JKXXAF
DOCUMENT TYPE:            Patent
LANGUAGE:                 Japanese
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.          KIND    DATE          APPLICATION NO.         DATE
     ---------------     ----    --------      --------------------    --------
     JP 2002355079        A2     20021210      JP 2002-69354           20020313
PRIORITY APPLN. INFO.:                         JP 2001-73183        A  20010314
                                               JP 2001-74993        A  20010315
                                               JP 2001-102519       A  20010330

L8   ANSWER 2 OF 9  CAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:         2001:319752  CAPLUS
DOCUMENT NUMBER:          134:331638
TITLE:                    Methods and compositions for treatment of keratoconus
                          using protease inhibitors
INVENTOR(S):              Quay, Steven C.
PATENT ASSIGNEE(S):       K-Quay Enterprises, Llc, USA
SOURCE:                   PCT Int. Appl., 68 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.          KIND    DATE          APPLICATION NO.         DATE
     ---------------     ----    --------      --------------------    --------
     WO 2001030380        A2     20010503      WO 2000-US29229         20001020
     WO 2001030380        A3     20011101
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
             HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
             LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,
             SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,
             YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
             DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
             CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
     EP 1231936           A2     20020821      EP 2000-972339          20001020
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, SI, LT, LV, FI, RO, MK, CY, AL
     US 6444791           B1     20020903      US 2000-695774          20001024
PRIORITY APPLN. INFO.:                         US 1999-161879P      P  19991027
                                               WO 2000-US29229      W  20001020
```

L8 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:570805 CAPLUS
DOCUMENT NUMBER: 131:281772
TITLE: The 20-kilodalton (kDa) human growth hormone (hGH) differs from the 22-kDa hGH in the effect on the human prolactin receptor
AUTHOR(S): Tsunekawa, Bunkichi; Wada, Mitsufumi; Ikeda, Miwa; Uchida, Hiroshi; Naito, Naokazu; Honjo, Masaru
CORPORATE SOURCE: Pharmaceuticals Section, Life Sciences Laboratory, Performance Materials R&D Center, Mitsui Chemicals, Inc., Mobara, 297-0017, Japan
SOURCE: Endocrinology (1999), 140(9), 3909-3918
CODEN: ENDOAO; ISSN: 0013-7227
PUBLISHER: Endocrine Society
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:493097 CAPLUS
DOCUMENT NUMBER: 131:138694
TITLE: Drug interactions with tobacco smoking: an update
AUTHOR(S): Zevin, Shoshana; Benowitz, Neal L.
CORPORATE SOURCE: Department of Internal Medicine, Shaare Zedek Medical Center, Jerusalem, Israel
SOURCE: Clinical Pharmacokinetics (1999), 36(6), 425-438
CODEN: CPKNDH; ISSN: 0312-5963
PUBLISHER: Adis International Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
REFERENCE COUNT: 129 THERE ARE 129 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1997:754883 CAPLUS
DOCUMENT NUMBER: 128:199
TITLE: Effects of orally administered capsaicin on hamster lung and liver enzyme activity
AUTHOR(S): Zhang, Zhuo; Teel, Robert W.
CORPORATE SOURCE: School Medicine, Loma Linda Univ., Loma Linda, CA, 92350, USA
SOURCE: Pharmacology Reviews and Communications (1997), 9(4), 247-258
CODEN: PHRCF6
PUBLISHER: Harwood Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

L8 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1990:417719 CAPLUS
DOCUMENT NUMBER: 113:17719
TITLE: A comparison of the α1-proteinase inhibitor methoxysuccinyl-Ala-Ala-Pro-Val-chloromethylketone and specific β-lactam inhibitors in an acute model of human polymorphonuclear leukocyte elastase-induced lung hemorrhage in the hamster
AUTHOR(S): Fletcher, Daniel S.; Osinga, Donald G.; Hand, Karen M.; Dellea, Pam S.; Ashe, Bonnie M.; Mumford, Richard A.; Davies, Philip; Hagmann, William; Finke, Paul E.; et al.
CORPORATE SOURCE: Merck Sharp and Dohme Res. Lab., Rahway, NJ, 07065,

```
                              USA
SOURCE:                       American Review of Respiratory Disease (1990), 141(3),
                              672-7
                              CODEN: ARDSBL; ISSN: 0003-0805
DOCUMENT TYPE:                Journal
LANGUAGE:                     English

L8   ANSWER 7 OF 9  CAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:             1986:513393  CAPLUS
DOCUMENT NUMBER:              105:113393
TITLE:                        Role of polymorphonuclear leukocytes in connective
                              tissue breakdown during the reverse passive Arthus
                              reaction
AUTHOR(S):                    Fletcher, Daniel S.; Osinga, Donald; Bonney, Robert J.
CORPORATE SOURCE:             Merck, Sharp and Dohme Res. Lab., Rahway, NJ, 07065,
                              USA
SOURCE:                       Biochemical Pharmacology (1986), 35(15), 2601-6
                              CODEN: BCPCA6; ISSN: 0006-2952
DOCUMENT TYPE:                Journal
LANGUAGE:                     English

L8   ANSWER 8 OF 9  CAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:             1983:503119  CAPLUS
DOCUMENT NUMBER:              99:103119
TITLE:                        In vitro and in vivo effects of chloramine T on rat
                              serum elastase inhibitor
AUTHOR(S):                    Lungarella, G.; Fonzi, L.; Benedetti, A.
CORPORATE SOURCE:             Ist. Patol. Gen., Univ. Siena, Siena, 53100, Italy
SOURCE:                       Clinical Respiratory Physiology (1983), 19(3), 273-7
                              CODEN: CRPHD4; ISSN: 0272-7587
DOCUMENT TYPE:                Journal
LANGUAGE:                     English

L8   ANSWER 9 OF 9  CAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:             1983:100737  CAPLUS
DOCUMENT NUMBER:              98:100737
TITLE:                        Pharmacokinetics of oxytetracycline and therapeutic
                              implications in veal calves
AUTHOR(S):                    Schifferli, D.; Galeazzi, R. L.; Nicolet, J.; Wanner,
                              M.
CORPORATE SOURCE:             Inst. Vet. Bacteriol., Univ. Berne, Bern, Switz.
SOURCE:                       Journal of Veterinary Pharmacology and Therapeutics
                              (1982), 5(4), 247-57
                              CODEN: JVPTD9; ISSN: 0140-7783
DOCUMENT TYPE:                Journal
LANGUAGE:                     English


=> d kwic 2

L8   ANSWER 2 OF 9  CAPLUS  COPYRIGHT 2006 ACS on STN
TI   Methods and compositions for treatment of keratoconus using protease
     inhibitors
AB   Compns. and methods for treating corneal diseases mediated by elevated
     protease activity include ocular administration of protease
     inhibitors.  One or more protease inhibitors selected
     from an aspartic, serine, cysteine, or metallo-protease inhibitor
     are administered to an ocular fluid, surface, or tissue,
     preferably by topical administration, to inhibit
     proteolytic activity associated with a corneal disease or condition, for
     example keratoconus.  Antiproteolytic formulations of the invention may
     include carriers that prolong the retention and/or enhance delivery of the
     protease inhibitor.  These formulations can also include other
     therapeutic agents such as antiinflammatory or antibiotic drugs.  In
     preferred aspects of the invention, antiproteolytic formulations are
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administered during periods of closed eye tear production Also provided within the invention are implant devices for corneal delivery of a protease inhibitor. For example, multiple test formulations were prepared by mixing a selected protease inhibitor (e.g, α2-macroglobulin or α1-antiprotease at a dose of 0.2-100 μg/mL) with various vehicles, including (1) a 0.81% (weight/volume) NaCl solution;. . .

ST topical protease inhibitor eye cornea disease

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(A, serine, amyloid; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Polymers, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(biocompatible; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Anti-inflammatory agents
Antibiotics
Antiviral agents
(combination with; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(gels, ophthalmic; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(implants, ocular; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Eye, disease
(keratopathy, keratoconus; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Polyesters, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(lactic acid-based; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(liposomes; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(microcapsules; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(microspheres; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(nanoparticles; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(ointments, ophthalmic, creams; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Polyethers, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(ortho ester group-containing; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Tear (ocular fluid)
(production of; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(prodrugs; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(solns., ophthalmic; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems

(suspensions, ophthalmic; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(sustained-release; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Latex
Preservatives
Stabilizing agents
(topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Collagens, biological studies
Polyanhydrides
Polyesters, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(topical; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Macroglobulins
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(α2-; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT 9001-92-7, Protease 37259-58-8, Serine protease 37353-41-6, Cysteine protease 78169-47-8, Aspartic protease 81669-70-7, Metalloprotease
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(inhibitors; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT 66-71-7, 1,10-Phenanthroline 329-98-6, Phenylmethyl sulfonyl fluoride 9041-92-3, α 1-Antiproteinase
9087-70-1, Aprotinin 39346-44-6, Inter-α-trypsin inhibitor
51798-45-9 55123-66-5, Leupeptin 91448-99-6, Cystatin C 122320-05-2, Secretory leukocyte protease inhibitor 124861-55-8, TIMP-2
133249-66-8, Elafin 138757-15-0, α2-Antiplasmin 140208-24-8, TIMP-1 141176-92-3, α1-Antichymotrypsin
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(topical compns. containing protease inhibitors for treatment of corneal diseases)

IT 9003-97-8, Polycarbophil 9004-38-0, Cellulose acetate hydrogen phthalate 24937-78-8, Ethylene vinyl acetate copolymer 26009-03-0, Poly(glycolic acid) 26023-30-3, Poly[oxy(1-methyl-2-oxo-1,2-ethanediyl)] 26100-51-6, Poly(lactic acid) 26124-68-5, Poly(glycolic acid) 26161-42-2
26811-96-1, Poly(L-lactic acid) 26917-25-9 106989-11-1, D-Lactic acid, homopolymer
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(topical compns. containing protease inhibitors for treatment of corneal diseases)

IT 37205-61-1, Proteinase inhibitor
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(β1-antigellagenase; topical compns. containing protease inhibitors for treatment of corneal diseases)

=> FIL REGISTRY

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	71.61	83.82

```
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)        SINCE FILE      TOTAL
                                                       ENTRY     SESSION
CA SUBSCRIBER PRICE                                    -0.75       -0.75

FILE 'REGISTRY' ENTERED AT 13:37:39 ON 20 SEP 2006
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STRUCTURE FILE UPDATES:    19 SEP 2006  HIGHEST RN 907944-91-6
DICTIONARY FILE UPDATES:   19 SEP 2006  HIGHEST RN 907944-91-6

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experimental property data in the original document.  For information
on property searching in REGISTRY, refer to:

http://www.cas.org/ONLINE/UG/regprops.html

=> S 9087-70-1/RN

L9            1 9087-70-1/RN

=> SET NOTICE 1 DISPLAY

NOTICE SET TO 1 U.S. DOLLAR FOR DISPLAY COMMAND
SET COMMAND COMPLETED

=> D L9 SQIDE 1-

YOU HAVE REQUESTED DATA FROM 1 ANSWERS - CONTINUE? Y/(N):y
THE ESTIMATED COST FOR THIS REQUEST IS 6.36 U.S. DOLLARS
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:y

L9   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   9087-70-1  REGISTRY
CN   Trypsin inhibitor, pancreatic basic (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Antikrein
CN   Antilysin
CN   Antilysine
CN   Aprotinin
CN   Basic pancreatic trypsin inhibitor
CN   Bayer A 128
CN   Bovine basic pancreatic trypsin inhibitor
CN   Bovine pancreatic trypsin inhibitor
CN   Bovine trypsin inhibitor
CN   BPTI
CN   BPTI trypsin inhibitor
CN   Fosten
CN   Kallikrein-trypsin inactivator
```

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CN   Kiker 52G
CN   Kir Richter
CN   Kunitz pancreatic trypsin inhibitor
CN   Kunitz protease inhibitor
CN   Kunitz trypsin inhibitor
CN   Kunitz-type inhibitor
CN   Kunitz-type proteinase inhibitor
CN   Kunitz-type trypsin inhibitor
CN   Onquinin
CN   Pancreatic basic trypsin inhibitor
CN   Pancreatic trypsin inhibitor
CN   Pancreatic trypsin inhibitor (Kunitz)
CN   Protease inhibitor, Kunitz type
CN   Repulson
CN   RP 9921
CN   Trasuylol
CN   Trasylol
CN   Trazinin
CN   Triazinin
CN   Trypsin inhibitor, Trasylol
CN   Trypsin-kallikrein inhibitor (Kunitz)
CN   Zymofren
DR   9004-04-0, 9039-77-4, 11005-72-4, 11132-77-7, 69431-34-1, 39283-52-8
MF   Unspecified
CI   COM, MAN
LC   STN Files:   ADISNEWS, AGRICOLA, ANABSTR, BIOSIS, BIOTECHNO, CA, CABA,
       CAPLUS, CBNB, CHEMCATS, CHEMLIST, CIN, CSCHEM, DDFU, DRUGU, EMBASE,
       IFICDB, IFIPAT, IFIUDB, IMSCOSEARCH, IMSDRUGNEWS, IMSRESEARCH, IPA,
       MEDLINE, MRCK*, MSDS-OHS, NAPRALERT, PHAR, PROMT, PS, RTECS*, SCISEARCH,
       TOXCENTER, USAN, USPAT2, USPATFULL, VTB
         (*File contains numerically searchable property data)
     Other Sources:   EINECS**, WHO
         (**Enter CHEMLIST File for up-to-date regulatory information)
DT.CA  CAplus document type:  Book; Conference; Dissertation; Journal; Patent;
       Report
RL.P   Roles from patents:  ANST (Analytical study); BIOL (Biological study);
       CMBI (Combinatorial study); MSC (Miscellaneous); OCCU (Occurrence); PREP
       (Preparation); PROC (Process); PRP (Properties); RACT (Reactant or
       reagent); USES (Uses)
RLD.P  Roles for non-specific derivatives from patents:  ANST (Analytical
       study); BIOL (Biological study); FORM (Formation, nonpreparative); PREP
       (Preparation); PROC (Process); PRP (Properties); RACT (Reactant or
       reagent); USES (Uses)
RL.NP  Roles from non-patents:  ANST (Analytical study); BIOL (Biological
       study); FORM (Formation, nonpreparative); MSC (Miscellaneous); OCCU
       (Occurrence); PREP (Preparation); PROC (Process); PRP (Properties); RACT
       (Reactant or reagent); USES (Uses); NORL (No role in record)
RLD.NP Roles for non-specific derivatives from non-patents:  ANST (Analytical
       study); BIOL (Biological study); FORM (Formation, nonpreparative); OCCU
       (Occurrence); PREP (Preparation); PROC (Process); PRP (Properties); RACT
       (Reactant or reagent); USES (Uses)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

**PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT**

              3959 REFERENCES IN FILE CA (1907 TO DATE)
               251 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
              3969 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> SET NOTICE LOGIN DISPLAY

NOTICE SET TO OFF  FOR DISPLAY COMMAND
SET COMMAND COMPLETED
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=>

=> d seq l9

L9   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2006 ACS on STN


=> d

L9   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   9087-70-1  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   Trypsin inhibitor, pancreatic basic (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Antikrein
CN   Antilysin
CN   Antilysine
CN   Aprotinin
CN   Basic pancreatic trypsin inhibitor
CN   Bayer A 128
CN   Bovine basic pancreatic trypsin inhibitor
CN   Bovine pancreatic trypsin inhibitor
CN   Bovine trypsin inhibitor
CN   BPTI
CN   BPTI trypsin inhibitor
CN   Fosten
CN   Kallikrein-trypsin inactivator
CN   Kiker 52G
CN   Kir Richter
CN   Kunitz pancreatic trypsin inhibitor
CN   Kunitz protease inhibitor
CN   Kunitz trypsin inhibitor
CN   Kunitz-type inhibitor
CN   Kunitz-type proteinase inhibitor
CN   Kunitz-type trypsin inhibitor
CN   Onquinin
CN   Pancreatic basic trypsin inhibitor
CN   Pancreatic trypsin inhibitor
CN   Pancreatic trypsin inhibitor (Kunitz)
CN   Protease inhibitor, Kunitz type
CN   Repulson
CN   RP 9921
CN   Trasuylol
CN   Trasylol
CN   Trazinin
CN   Triazinin
CN   Trypsin inhibitor, Trasylol
CN   Trypsin-kallikrein inhibitor (Kunitz)
CN   Zymofren
DR   9004-04-0, 9039-77-4, 11005-72-4, 11132-77-7, 69431-34-1, 39283-52-8
MF   Unspecified
CI   COM, MAN
LC   STN Files:   ADISNEWS, AGRICOLA, ANABSTR, BIOSIS, BIOTECHNO, CA, CABA,
       CAPLUS, CBNB, CHEMCATS, CHEMLIST, CIN, CSCHEM, DDFU, DRUGU, EMBASE,
       IFICDB, IFIPAT, IFIUDB, IMSCOSEARCH, IMSDRUGNEWS, IMSRESEARCH, IPA,
       MEDLINE, MRCK*, MSDS-OHS, NAPRALERT, PHAR, PROMT, PS, RTECS*, SCISEARCH,
       TOXCENTER, USAN, USPAT2, USPATFULL, VTB
         (*File contains numerically searchable property data)
     Other Sources:   EINECS**, WHO
         (**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

**PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT**
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          3959 REFERENCES IN FILE CA (1907 TO DATE)
           251 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
          3969 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> file caplus
COST IN U.S. DOLLARS                              SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     5.56      89.38

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)        SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                     0.00      -0.75

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FILE COVERS 1907 - 20 Sep 2006  VOL 145 ISS 13
FILE LAST UPDATED: 19 Sep 2006  (20060919/ED)

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     (FILE 'HOME' ENTERED AT 13:31:23 ON 20 SEP 2006)

     FILE 'REGISTRY' ENTERED AT 13:31:29 ON 20 SEP 2006
                E "SERPINA"/CN 25
L1            1 S E23

     FILE 'CAPLUS' ENTERED AT 13:32:41 ON 20 SEP 2006
L2            1 S L1
L3         1038 S 2A2 OR AI119734 OR ALPHA-1 ANTIPROTEINASE OR ALPHA-1 ANTIPROT
L4          866 S L3 NOT PY>2002
L5      1863727 S INHIBIT?
L6          147 S L5 AND L4
L7      1277182 S CONTACT? OR ADMINIST?
L8            9 S L7 AND L6

     FILE 'REGISTRY' ENTERED AT 13:37:39 ON 20 SEP 2006
L9            1 S 9087-70-1/RN
                SET NOTICE 1 DISPLAY
                SET NOTICE LOGIN DISPLAY

     FILE 'CAPLUS' ENTERED AT 13:40:11 ON 20 SEP 2006

=> d 18 2 ibib bs kwic
'BS' IS NOT A VALID FORMAT FOR FILE 'CAPLUS'
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The following are valid formats:

ABS --------- GI and AB
ALL --------- BIB, AB, IND, RE
APPS -------- AI, PRAI
BIB --------- AN, plus Bibliographic Data and PI table (default)
CAN --------- List of CA abstract numbers without answer numbers
CBIB -------- AN, plus Compressed Bibliographic Data
CLASS ------- IPC, NCL, ECLA, FTERM
DALL -------- ALL, delimited (end of each field identified)
DMAX -------- MAX, delimited for post-processing
FAM --------- AN, PI and PRAI in table, plus Patent Family data
FBIB -------- AN, BIB, plus Patent FAM
IND --------- Indexing data
IPC --------- International Patent Classifications
MAX --------- ALL, plus Patent FAM, RE
PATS -------- PI, SO
SAM --------- CC, SX, TI, ST, IT
SCAN -------- CC, SX, TI, ST, IT (random display, no answer numbers;
              SCAN must be entered on the same line as the DISPLAY,
              e.g., D SCAN or DISPLAY SCAN)
STD --------- BIB, CLASS

IABS -------- ABS, indented with text labels
IALL -------- ALL, indented with text labels
IBIB -------- BIB, indented with text labels
IMAX -------- MAX, indented with text labels
ISTD -------- STD, indented with text labels

OBIB -------- AN, plus Bibliographic Data (original)
OIBIB ------- OBIB, indented with text labels

SBIB -------- BIB, no citations
SIBIB ------- IBIB, no citations

HIT --------- Fields containing hit terms
HITIND ------ IC, ICA, ICI, NCL, CC and index field (ST and IT)
              containing hit terms
HITRN ------- HIT RN and its text modification
HITSTR ------ HIT RN, its text modification, its CA index name, and
              its structure diagram
HITSEQ ------ HIT RN, its text modification, its CA index name, its
              structure diagram, plus NTE and SEQ fields
FHITSTR ----- First HIT RN, its text modification, its CA index name, and
              its structure diagram
FHITSEQ ----- First HIT RN, its text modification, its CA index name, its
              structure diagram, plus NTE and SEQ fields
KWIC -------- Hit term plus 20 words on either side
OCC --------- Number of occurrence of hit term and field in which it occurs

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codes.  For a list of the display field codes, enter HELP DFIELDS at
an arrow prompt (=>).  Examples of formats include: TI; TI,AU; BIB,ST;
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specification.

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to view a specified Accession Number.
ENTER DISPLAY FORMAT (BIB):end

=> d 18 2 ibib abs kwic
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L8 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:319752 CAPLUS
DOCUMENT NUMBER: 134:331638
TITLE: Methods and compositions for treatment of keratoconus using protease inhibitors
INVENTOR(S): Quay, Steven C.
PATENT ASSIGNEE(S): K-Quay Enterprises, Llc, USA
SOURCE: PCT Int. Appl., 68 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 2001030380	A2	20010503	WO 2000-US29229		20001020
WO 2001030380	A3	20011101			
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG					
EP 1231936	A2	20020821	EP 2000-972339		20001020
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL					
US 6444791	B1	20020903	US 2000-695774		20001024
PRIORITY APPLN. INFO.:			US 1999-161879P	P	19991027
			WO 2000-US29229	W	20001020

AB Compns. and methods for treating corneal diseases mediated by elevated protease activity include ocular administration of protease inhibitors. One or more protease inhibitors selected from an aspartic, serine, cysteine, or metallo-protease inhibitor are administered to an ocular fluid, surface, or tissue, preferably by topical administration, to inhibit proteolytic activity associated with a corneal disease or condition, for example keratoconus. Antiproteolytic formulations of the invention may include carriers that prolong the retention and/or enhance delivery of the protease inhibitor. These formulations can also include other therapeutic agents such as antiinflammatory or antibiotic drugs. In preferred aspects of the invention, antiproteolytic formulations are administered during periods of closed eye tear production Also provided within the invention are implant devices for corneal delivery of a protease inhibitor. For example, multiple test formulations were prepared by mixing a selected protease inhibitor (e.g, α2-macroglobulin or α1-antiprotease at a dose of 0.2-100 μg/mL) with various vehicles, including (1) a 0.81% (weight/volume) NaCl solution; (2) 0.81% (weight/volume) NaCl and 4.5% (weight/volume) polycarbophil in a polycarbophil (PCP) formulation; and (3) 0.81% (weight/volume) NaCl and 4.5% (weight/volume) polycarbophil adjusted to pH 7.5 with 10N NaOH in a pH adjusted PCP formulation. The buffer capacity of the PCP formulation is calculated to be 0.01, comparable to that of rabbit tears.

TI Methods and compositions for treatment of keratoconus using protease inhibitors

AB Compns. and methods for treating corneal diseases mediated by elevated protease activity include ocular administration of protease inhibitors. One or more protease inhibitors selected from an aspartic, serine, cysteine, or metallo-protease inhibitor are administered to an ocular fluid, surface, or tissue, preferably by topical administration, to inhibit

proteolytic activity associated with a corneal disease or condition, for example keratoconus. Antiproteolytic formulations of the invention may include carriers that prolong the retention and/or enhance delivery of the protease inhibitor. These formulations can also include other therapeutic agents such as antiinflammatory or antibiotic drugs. In preferred aspects of the invention, antiproteolytic formulations are administered during periods of closed eye tear production Also provided within the invention are implant devices for corneal delivery of a protease inhibitor. For example, multiple test formulations were prepared by mixing a selected protease inhibitor (e.g, α2-macroglobulin or α1-antiprotease at a dose of 0.2-100 μg/mL) with various vehicles, including (1) a 0.81% (weight/volume) NaCl solution; (2) 0.81% (weight/volume) NaCl and 4.5% (weight/volume)
polycarbophil in a
polycarbophil (PCP) formulation; and (3) 0.81% (weight/volume) NaCl and 4.5% (weight/volume) polycarbophil adjusted to pH 7.5 with 10N NaOH in a pH adjusted PCP formulation. The buffer capacity of the PCP formulation is calculated to be 0.01, comparable to that of rabbit tears.

ST topical protease inhibitor eye cornea disease

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(A, serine, amyloid; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Polymers, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(biocompatible; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Anti-inflammatory agents
Antibiotics
Antiviral agents
(combination with; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(gels, ophthalmic; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(implants, ocular; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Eye, disease
(keratopathy, keratoconus; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Polyesters, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(lactic acid-based; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(liposomes; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(microcapsules; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(microspheres; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(nanoparticles; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Drug delivery systems
(ointments, ophthalmic, creams; topical compns. containing protease inhibitors for treatment of corneal diseases)

IT Polyethers, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(ortho ester group-containing; topical compns. containing protease

inhibitors for treatment of corneal diseases)
IT Tear (ocular fluid)
(production of; topical compns. containing protease inhibitors for treatment of corneal diseases)
IT Drug delivery systems
(prodrugs; topical compns. containing protease inhibitors for treatment of corneal diseases)
IT Drug delivery systems
(solns., ophthalmic; topical compns. containing protease inhibitors for treatment of corneal diseases)
IT Drug delivery systems
(suspensions, ophthalmic; topical compns. containing protease inhibitors for treatment of corneal diseases)
IT Drug delivery systems
(sustained-release; topical compns. containing protease inhibitors for treatment of corneal diseases)
IT Latex
Preservatives
Stabilizing agents
(topical compns. containing protease inhibitors for treatment of corneal diseases)
IT Collagens, biological studies
Polyanhydrides
Polyesters, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(topical compns. containing protease inhibitors for treatment of corneal diseases)
IT Drug delivery systems
(topical; topical compns. containing protease inhibitors for treatment of corneal diseases)
IT Macroglobulins
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(α2-; topical compns. containing protease inhibitors for treatment of corneal diseases)
IT 9001-92-7, Protease 37259-58-8, Serine protease 37353-41-6, Cysteine protease 78169-47-8, Aspartic protease 81669-70-7, Metalloprotease
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(inhibitors; topical compns. containing protease inhibitors for treatment of corneal diseases)
IT 66-71-7, 1,10-Phenanthroline 329-98-6, Phenylmethyl sulfonyl fluoride
9041-92-3, α 1-Antiproteinase
9087-70-1, Aprotinin 39346-44-6, Inter-α-trypsin inhibitor
51798-45-9 55123-66-5, Leupeptin 91448-99-6, Cystatin C 122320-05-2, Secretory leukocyte protease inhibitor 124861-55-8, TIMP-2
133249-66-8, Elafin 138757-15-0, α2-Antiplasmin 140208-24-8, TIMP-1 141176-92-3, α1-Antichymotrypsin
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(topical compns. containing protease inhibitors for treatment of corneal diseases)
IT 9003-97-8, Polycarbophil 9004-38-0, Cellulose acetate hydrogen phthalate
24937-78-8, Ethylene vinyl acetate copolymer 26009-03-0, Poly(glycolic acid) 26023-30-3, Poly[oxy(1-methyl-2-oxo-1,2-ethanediyl)] 26100-51-6, Poly(lactic acid) 26124-68-5, Poly(glycolic acid) 26161-42-2
26811-96-1, Poly(L-lactic acid) 26917-25-9 106989-11-1, D-Lactic acid, homopolymer
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(topical compns. containing protease inhibitors for treatment of corneal diseases)
IT 37205-61-1, Proteinase inhibitor
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES

```
     (Uses)
        (β1-antigellagenase; topical compns. containing protease
        inhibitors for treatment of corneal diseases)


=> d his

     (FILE 'HOME' ENTERED AT 13:31:23 ON 20 SEP 2006)

     FILE 'REGISTRY' ENTERED AT 13:31:29 ON 20 SEP 2006
                E "SERPINA"/CN 25
L1            1 S E23

     FILE 'CAPLUS' ENTERED AT 13:32:41 ON 20 SEP 2006
L2            1 S L1
L3         1038 S 2A2 OR AI119734 OR ALPHA-1 ANTIPROTEINASE OR ALPHA-1 ANTIPROT
L4          866 S L3 NOT PY>2002
L5      1863727 S INHIBIT?
L6          147 S L5 AND L4
L7      1277182 S CONTACT? OR ADMINIST?
L8            9 S L7 AND L6

     FILE 'REGISTRY' ENTERED AT 13:37:39 ON 20 SEP 2006
L9            1 S 9087-70-1/RN
                SET NOTICE 1 DISPLAY
                SET NOTICE LOGIN DISPLAY

     FILE 'CAPLUS' ENTERED AT 13:40:11 ON 20 SEP 2006

=> s Serine protease inhibitor 2A or Serine protease inhibitor A3G or Serpin 2A or
Serpin A3G or Spi2-1 or Spi2A
        109032 SERINE
          1771 SERINES
        109700 SERINE
                 (SERINE OR SERINES)
         95580 PROTEASE
         35069 PROTEASES
        111486 PROTEASE
                 (PROTEASE OR PROTEASES)
        518101 INHIBITOR
        525022 INHIBITORS
        817793 INHIBITOR
                 (INHIBITOR OR INHIBITORS)
         37227 2A
             3 SERINE PROTEASE INHIBITOR 2A
                 (SERINE(W)PROTEASE(W)INHIBITOR(W)2A)
        109032 SERINE
          1771 SERINES
        109700 SERINE
                 (SERINE OR SERINES)
         95580 PROTEASE
         35069 PROTEASES
        111486 PROTEASE
                 (PROTEASE OR PROTEASES)
        518101 INHIBITOR
        525022 INHIBITORS
        817793 INHIBITOR
                 (INHIBITOR OR INHIBITORS)
            33 A3G
             0 SERINE PROTEASE INHIBITOR A3G
                 (SERINE(W)PROTEASE(W)INHIBITOR(W)A3G)
          2144 SERPIN
           940 SERPINS
          2348 SERPIN
                 (SERPIN OR SERPINS)
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        37227 2A
            7 SERPIN 2A
                (SERPIN(W)2A)
         2144 SERPIN
          940 SERPINS
         2348 SERPIN
                (SERPIN OR SERPINS)
           33 A3G
            1 SERPIN A3G
                (SERPIN(W)A3G)
          123 SPI2
      8829298 1
           12 SPI2-1
                (SPI2(W)1)
            6 SPI2A
L10        24 SERINE PROTEASE INHIBITOR 2A OR SERINE PROTEASE INHIBITOR A3G
              OR SERPIN 2A OR SERPIN A3G OR SPI2-1 OR SPI2A

=> s l10 not py>2002
      4356482 PY>2002
L11        15 L10 NOT PY>2002

=> s l11 and apop?
       142320 APOP?
L12         0 L11 AND APOP?

=> d his

     (FILE 'HOME' ENTERED AT 13:31:23 ON 20 SEP 2006)

     FILE 'REGISTRY' ENTERED AT 13:31:29 ON 20 SEP 2006
                E "SERPINA"/CN 25
L1            1 S E23

     FILE 'CAPLUS' ENTERED AT 13:32:41 ON 20 SEP 2006
L2            1 S L1
L3         1038 S 2A2 OR AI119734 OR ALPHA-1 ANTIPROTEINASE OR ALPHA-1 ANTIPROT
L4          866 S L3 NOT PY>2002
L5      1863727 S INHIBIT?
L6          147 S L5 AND L4
L7      1277182 S CONTACT? OR ADMINIST?
L8            9 S L7 AND L6

     FILE 'REGISTRY' ENTERED AT 13:37:39 ON 20 SEP 2006
L9            1 S 9087-70-1/RN
                SET NOTICE 1 DISPLAY
                SET NOTICE LOGIN DISPLAY

     FILE 'CAPLUS' ENTERED AT 13:40:11 ON 20 SEP 2006
L10          24 S SERINE PROTEASE INHIBITOR 2A OR SERINE PROTEASE INHIBITOR A3G
L11          15 S L10 NOT PY>2002
L12           0 S L11 AND APOP?

=> s l12 and l7
L13          0 L12 AND L7

=> s l11 and l7
L14          2 L11 AND L7

=> d ibib 1-2

L14  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:         2000:685505  CAPLUS
DOCUMENT NUMBER:          133:291352
TITLE:                    Hepatic growth hormone signaling in the late gestation
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                              fetal rat
AUTHOR(S):                    Phornphutkul, Chanika; Frick, G. Peter; Goodman, H.
                              Maurice; Berry, Susan A.; Gruppuso, Philip A.
CORPORATE SOURCE:             Department of Pediatrics, Brown University and Rhode
                              Island Hospital, Providence, RI, 02903, USA
SOURCE:                       Endocrinology (2000), 141(10), 3527-3533
                              CODEN: ENDOAO; ISSN: 0013-7227
PUBLISHER:                    Endocrine Society
DOCUMENT TYPE:                Journal
LANGUAGE:                     English
REFERENCE COUNT:              43    THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS
                                    RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:             1999:570805  CAPLUS
DOCUMENT NUMBER:              131:281772
TITLE:                        The 20-kilodalton (kDa) human growth hormone (hGH)
                              differs from the 22-kDa hGH in the effect on the human
                              prolactin receptor
AUTHOR(S):                    Tsunekawa, Bunkichi; Wada, Mitsufumi; Ikeda, Miwa;
                              Uchida, Hiroshi; Naito, Naokazu; Honjo, Masaru
CORPORATE SOURCE:             Pharmaceuticals Section, Life Sciences Laboratory,
                              Performance Materials R&D Center, Mitsui Chemicals,
                              Inc., Mobara, 297-0017, Japan
SOURCE:                       Endocrinology (1999), 140(9), 3909-3918
                              CODEN: ENDOAO; ISSN: 0013-7227
PUBLISHER:                    Endocrine Society
DOCUMENT TYPE:                Journal
LANGUAGE:                     English
REFERENCE COUNT:              48    THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS
                                    RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT


=> d kwic 1-2

L14  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2006 ACS on STN
AB   . . . phosphorylation of these signaling proteins.  To confirm the
     relevance of these findings to the in vivo situation, GH was directly
     administered by i.p. injection to E19 fetal and adult rats.  In
     both cases, tyrosine phosphorylation of STAT5 was markedly and rapidly
     induced.  Finally, transfection of E19 fetal hepatocytes with
     GH-responsive reporter elements [Spi2.1(-275/+85)-CAT
     and 8xGHRE-TKCAT] demonstrated intact transcriptional regulation.  The
     authors' data indicate that GHr abundance and activity as well as
     downstream GH. . .

L14  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2006 ACS on STN
AB   . . . effect of 20K-hGH on activating hPRLR in the absence of Zn2+ was
     confirmed in the rat serine protease inhibitor 2.1 (Spi2.
     1) gene promoter activation and JAK2/Stat5 tyrosine
     phosphorylation in CHO-hPRLR.  In addition, in human breast cancer cell
     T-47D, 20K-hGH was proved. . .  for hPRLR than 22K-hGH in the human
     body; therefore 20K-hGH may alleviate the hPRLR-mediated side-effects such
     as breast cancer when administered to human body.


=> file his
'HIS' IS NOT A VALID FILE NAME
SESSION CONTINUES IN FILE 'CAPLUS'
Enter "HELP FILE NAMES" at an arrow prompt (=>) for a list of files
that are available.  If you have requested multiple files, you can
specify a corrected file name or you can enter "IGNORE" to continue
accessing the remaining file names entered.

=> d his
```

```
     (FILE 'HOME' ENTERED AT 13:31:23 ON 20 SEP 2006)

     FILE 'REGISTRY' ENTERED AT 13:31:29 ON 20 SEP 2006
                E "SERPINA"/CN 25
L1            1 S E23

     FILE 'CAPLUS' ENTERED AT 13:32:41 ON 20 SEP 2006
L2            1 S L1
L3         1038 S 2A2 OR AI119734 OR ALPHA-1 ANTIPROTEINASE OR ALPHA-1 ANTIPROT
L4          866 S L3 NOT PY>2002
L5      1863727 S INHIBIT?
L6          147 S L5 AND L4
L7      1277182 S CONTACT? OR ADMINIST?
L8            9 S L7 AND L6

     FILE 'REGISTRY' ENTERED AT 13:37:39 ON 20 SEP 2006
L9            1 S 9087-70-1/RN
                SET NOTICE 1 DISPLAY
                SET NOTICE LOGIN DISPLAY

     FILE 'CAPLUS' ENTERED AT 13:40:11 ON 20 SEP 2006
L10          24 S SERINE PROTEASE INHIBITOR 2A OR SERINE PROTEASE INHIBITOR A3G
L11          15 S L10 NOT PY>2002
L12           0 S L11 AND APOP?
L13           0 S L12 AND L7
L14           2 S L11 AND L7

=> s l11 and cell
       2111300 CELL
       1846211 CELLS
       2791311 CELL
                 (CELL OR CELLS)
L15          11 L11 AND CELL

=> d ibib kwic 1-11

L15  ANSWER 1 OF 11  CAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:         2002:183325  CAPLUS
DOCUMENT NUMBER:          136:308498
TITLE:                    Serpin 2a is induced in activated
                          macrophages and conjugates to a ubiquitin homolog
AUTHOR(S):                Hamerman, Jessica A.; Hayashi, Fumitaka; Schroeder,
                          Lea A.; Gygi, Steven P.; Haas, Arthur L.; Hampson,
                          Lynne; Coughlin, Paul; Aebersold, Ruedi; Aderem, Alan
CORPORATE SOURCE:         Department of Immunology, University of Washington,
                          Seattle, WA, 98185, USA
SOURCE:                   Journal of Immunology (2002), 168(5), 2415-2423
                          CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER:                American Association of Immunologists
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
REFERENCE COUNT:          52    THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT
TI   Serpin 2a is induced in activated macrophages and
     conjugates to a ubiquitin homolog
AB   After i.p. infection of mice with the intracellular bacterium
     Mycobacterium bovis bacillus Calmette-Guerin, macrophages recovered from
     the peritoneal cavity display classical signs of immune activation.  The
     authors have identified a member of the serine protease inhibitor (serpin)
     family which is highly induced in macrophages during bacillus
     Calmette-Guerin infection.  Serpin 2a (spi2a
     ) expression is also induced in macrophages in vivo during infection with
     Salmonella typhimurium and Listeria monocytogenes, and in vitro by a
     variety of bacteria and bacterial products.  The cytokine IFN-γ also
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induces spi2a expression in macrophages, and this induction is synergistic with bacterial products. The authors also demonstrate here that a ubiquitin homolog, IFN-stimulated gene of 15-kDa (ISG15), is strongly induced during in vitro and in vivo activation of macrophages and that it conjugates to spi2a in activated macrophages. The ISG15-spi2a conjugates were identified by tandem mass spectrometry and contained spi2a conjugated to either one or two mols. of ISG15. Whereas spi2a was induced by either bacterial products or IFN-γ, ISG15 was induced only by bacterial products. Although many protein targets have been described for ubiquitin conjugation, spi2a is the first ISG15-modified protein to be reported. Macrophage activation is accompanied by the activation of a variety of proteases. It is of interest that a member of the serine protease inhibitor family is concomitantly induced and modified by a ubiquitin-like protein.

ST serpin 2a conjugate ISG15 protein macrophage activation

IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ISG15 conjugates, with serpin 2a; expression in macrophages activated by bacterial products)

IT Macrophage
(activation; serpin 2a expression and conjugation to ISG15 in)

IT Mycobacterium BCG
(bacterial infection induces serpin 2a expression and conjugation to ISG15 in activated macrophages)

IT Infection
(bacterial; serpin 2a expression and conjugation to ISG15 in macrophages in)

IT Cell activation
(macrophage; serpin 2a expression and conjugation to ISG15 in)

IT Listeria monocytogenes
Salmonella typhimurium
(serpin 2a expression in macrophages is induced by infection with)

IT Interferons
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(γ; serpin 2a expression in macrophages is induced by)

IT 186148-63-0, Serpin 2a
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(expression in macrophage activation)

IT 186148-63-0D, Serpin 2a, ISG15 conjugates
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(expression in macrophages activated by bacterial products)

L15 ANSWER 2 OF 11 CAPLUS

ACCESSION NUMBER: 2001:801587 CAPLUS
DOCUMENT NUMBER: 137:1347
TITLE: A minimal serpin promoter with high activity in haematopoietic progenitors and activated T cells
AUTHOR(S): Hampson, Lynne; Hampson, Ian N.; Babichuk, Charolyn K.; Cotter, Laura; Bleackley, R. Chris; Dexter, T. Michael; Cross, Michael A.
CORPORATE SOURCE: CRC Department of Hematopoietic Cell and Gene Therapeutics, Paterson Institute for Cancer Research, Manchester, M20 4BX, UK
SOURCE: Hematology Journal (2001), 2(3), 150-160
CODEN: HJEOBZ; ISSN: 1466-4860
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI A minimal serpin promoter with high activity in haematopoietic progenitors and activated T cells

AB The serine protease inhibitor Serpin 2A is highly expressed in ex vivo bipotent granulocyte/macrophage progenitor cells and in cultured myeloid stem cells. The gene undergoes rapid down-regulation as these cells are induced to differentiate, and constitutive expression in cultured myeloid stem cells retards maturation. Serpin 2A is also expressed in T cells as a consequence of activation. The authors now report anal. of the upstream regulatory elements that control Serpin 2A transcription. Primer extension and rapid amplification of cDNA ends were used to map the transcription start site of the Serpin 2A gene, and a 1.2 Kb genomic upstream fragment cloned and sequenced. Promoter activity and protein binding of deletion and site-directed mutant constructs were analyzed by transient transfection and by electrophoretic mobility shift assays. A minimal promoter fragment was identified with high activity dependent on NF-κB and Moloney murine leukemia enhancer factor LVa binding sites in both myeloid stem cells and activated T cells. NF-κB was shown to be the main DNA binding protein in T cells, whereas that in hematopoietic stem cells appears to be novel. Serpin 2A promoter activity in T cells is due predominantly to NF-κB binding to its consensus site. Activity in hematopoietic stem cells appears to be mediated by a novel protein, which recognizes the NF-κB consensus only in the context of flanking sequences. This concise regulatory element may be of potential value in gene therapeutic applications.

ST Serpin 2A promoter sequence NFkappaB site LVa site mouse; hematopoietic progenitor activated T cell Serpin 2A promoter mouse

IT Animal cell line
(FDCPmix; mouse minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells)

IT Genetic element
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(LVa site; of minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells)

IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(LVa; minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells with binding site for)

IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(NF-κB (nuclear factor of κ light chain gene enhancer in B-cells); minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells activation by)

IT Genetic element
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(NF-κB site; of minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells)

IT Hematopoietic precursor cell
T cell (lymphocyte)
(minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells)

IT Gene, animal
Promoter (genetic element)
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL

(Biological study)
(minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells)

IT Mus
(mouse minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells)

IT DNA sequences
(of mouse minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells)

IT Genetic element
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(tsp (transcription start point); of minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells)

IT 186148-63-0, Serpin 2A
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells)

IT 432831-00-0
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(nucleotide sequence; minimal Serpin 2A promoter with high activity in hematopoietic progenitors and activated T cells)

L15 ANSWER 3 OF 11 CAPLUS

ACCESSION NUMBER: 2001:343723 CAPLUS
DOCUMENT NUMBER: 135:56276
TITLE: Cellular activities of 20K- and 22K-hGH do not necessarily correlate with their binding affinities for rat GH receptor
AUTHOR(S): Ikeda, M.; Matsumoto, K.; Uchida, H.; Naito, N.; Tsunekawa, B.; Wada, M.; Honjo, M.
CORPORATE SOURCE: Pharmaceuticals Section, Life Science Laboratories, Mitsui Chemicals, Inc., Chiba, 297-0017, Japan
SOURCE: Hormone Research (2001), Volume Date 2000, 54(3), 136-142
CODEN: HRMRA3; ISSN: 0301-0163
PUBLISHER: S. Karger AG
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB Even though 20K human growth hormone (20K-hGH) has 3-10% binding affinity for the rat liver and adipose tissue microsomes as compared to 22K-hGH, it was also reported that 20K-hGH has the same potency as 22K-hGH in the hypophysectomized rat weight gain assay. In order to investigate the reason why such controversial data exist, the authors have studied 20K- and 22K-hGH using the rat GH receptor extracellular domain (rGHR-ECD) and full-length rGHR. When the authors examined the complex formation of rGHR-ECD with 20K- and 22K-hGH in gel filtration assay, 20K-hGH formed no complex while 22K-hGH formed a 1:1 complex. Next, rGHR cDNA was introduced into Ba/F3 cells and CHO-K1 cells, and stable transfectants (Ba/F3-rGHR and CHO-rGHR) were established. In the proliferation of Ba/F3-rGHR cells, 20K-hGH had 10-fold lower activity than 22K-hGH, which is consistent with their affinities for rGHR. But surprisingly, in the Spi2.1 gene promoter activation in CHO-rGHR cells, 20K- and 22K-hGH had the same activity, which was found not only in stable CHO-rGHR clones but also in CHO-K1 cells transiently expressing rGHR. In conclusion, these results indicate that cellular activities of 20K- and 22K-hGH do not necessarily correlate with their binding affinities for rGHR.

IT Cell proliferation
(cellular activities of 20K- and 22K-hGH do not necessarily correlate with binding affinities for rat GH receptor)

IT Promoter (genetic element)
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(of Spi2.1 gene; cellular activities of 20K- and 22K-hGH do not necessarily correlate with binding affinities for rat GH receptor)

L15 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2000:740381 CAPLUS
DOCUMENT NUMBER: 134:16155
TITLE: Chemical-induced hippocampal neurodegeneration and elevations in TNFα, TNFβ, IL-1α, IP-10, and MCP-1 mRNA in osteopetrotic (op/op) mice
AUTHOR(S): Bruccoleri, Alessandra; Harry, G. Jean
CORPORATE SOURCE: Neurotoxicology Group, National Institute of Environmental Health Sciences, Research Triangle Park, NC, 27709, USA
SOURCE: Journal of Neuroscience Research (2000), 62(1), 146-155
CODEN: JNREDK; ISSN: 0360-4012
PUBLISHER: Wiley-Liss, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB The osteopetrotic (op/op) mouse, deficient in biol. active colony stimulating factor 1 (CSF-1), was used to examine the role of microglia in chemical-induced trauma. Op/op mice and normal phenotype littermates (non-op/op) received an acute i.p. injection of the hippocampal toxicant, trimethyltin hydroxide (TMT; 1.5 or 2.0 mg/kg). At 2.0 mg/kg, both mice displayed severe degeneration of dentate granule neurons. At 1.5 mg/kg, non-op/op mice showed a limited punctate pattern of neuronal death while op/op mice showed prominent neuronal death. TMT-induced astrocyte reactivity was similar in both groups. RNase protection assays were conducted on hippocampal tissue at 24 h post-TMT. Elevations were seen in mRNA levels for the host response genes: intercellular cell adhesion mol. (ICAM-1; non-op/op 80%, op/op 85%), the protease inhibitor EB22 (non-op/op 60%, op/op 300%), and glial fibrillary acidic protein (GFAP; non-op/op 300%, op/op 480%) within 24 h. Macrophage-1 antigen (Mac-1) mRNA levels were lower in all op/op mice and were not induced by TMT exposure. Macrophage inflammatory protein (MIP)-1α and MIP-1β mRNA levels were elevated in non-op/op mice while mRNA levels for interferon inducible protein (IP-10) and monocyte chemoattractant protein (MCP-1) were elevated in op/op mice. Tumor necrosis factor alpha (TNFα) mRNA levels were significantly elevated in both non-op/op (100%) and op/op (600%) mice. TNFβ mRNA levels in op/op mice were elevated 200% and interleukin 1alpha (IL-1α) 150%. Reverse transcriptase polymerase chain reaction (RT-PCR) showed a TMT-induced elevation in INFα and INFβ mRNA levels and no elevation of INFγ. MRNA levels of the CSF-1 receptor, c-fms, were unaltered.

IT Cell adhesion molecules
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ICAM-1 (intercellular adhesion mol. 1); chemical-induced hippocampal neurodegeneration and elevations in host response genes enconding TNFα, TNFβ, IL-1α, IP-10, MCP-1 and other proteins in osteopetrotic (op/op) mice)

IT Cell death
(neuron; chemical-induced hippocampal neurodegeneration and elevations in host response genes enconding TNFα, TNFβ, IL-1α, IP-10, MCP-1 and other proteins in osteopetrotic (op/op) mice)

IT 186148-63-0, Serpin 2A
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(chemical-induced hippocampal neurodegeneration and elevations in host response genes enconding TNFα, TNFβ, IL-1α, IP-10, MCP-1 and other proteins in osteopetrotic (op/op) mice)

L15 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1999:570805 CAPLUS
DOCUMENT NUMBER: 131:281772
TITLE: The 20-kilodalton (kDa) human growth hormone (hGH) differs from the 22-kDa hGH in the effect on the human prolactin receptor

AUTHOR(S): Tsunekawa, Bunkichi; Wada, Mitsufumi; Ikeda, Miwa; Uchida, Hiroshi; Naito, Naokazu; Honjo, Masaru
CORPORATE SOURCE: Pharmaceuticals Section, Life Sciences Laboratory, Performance Materials R&D Center, Mitsui Chemicals, Inc., Mobara, 297-0017, Japan

SOURCE: Endocrinology (1999), 140(9), 3909-3918
CODEN: ENDOAO; ISSN: 0013-7227
PUBLISHER: Endocrine Society
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB Previously we have demonstrated that 20-kDa human GH (20K-hGH) is a full agonist for hGH receptor (hGHR) even though its complex formation with hGHR and hGH-binding protein differs from that of 22-kDa human GH (22K-hGH). In this study, we focused on the effect of 20K-hGH on human PRL receptor (hPRLR). To elucidate the effects of 20K-hGH on hPRLR and compare them with those of 22K-hGH, we prepared two cells stably expressing full-length hPRLR, Ba/F3-hPRLR and CHO-hPRLR. In the proliferation of Ba/F3-hPRLR cells, which can grow in a dose-response to lactogenic hormones, both 20K- and 22K-hGH exhibited bell-shaped curves in the absence of exogenous zinc ion (Zn2+); however, the curve of 20K-hGH was shifted to a 10-fold higher concentration than that of 22K-hGH in view of EC50 value (the EC50 of 20K- and 22K-hGH were 15 nM, and 1.5 nM, resp.). Addition of Zn2+ up to 25 μM increased the activities of both 20K- and 22K-hGH; however, the enhancement by Zn2+ was greater in 20K-hGH than in 22K-hGH, thereby the activities of both hGH isoforms reached the same level at 25 μM Zn2+. Nevertheless, in the presence of 0.25-1 μM free Zn2+, which is equal in human serum, the activity of 20K-hGH was still lower than that of 22K-hGH. The modest effect of 20K-hGH on activating hPRLR in the absence of Zn2+ was confirmed in the rat serine protease inhibitor 2.1 (Spi2.1) gene promoter activation and JAK2/Stat5 tyrosine phosphorylation in CHO-hPRLR. In addition, in human breast cancer cell T-47D, 20K-hGH was proved to stimulate Stat5 tyrosine phosphorylation to much lower degree than 22K-hGH via not hGHR but hPRLR. Taken together, our data suggest that 20K-hGH may be a weaker agonist for hPRLR than 22K-hGH in the human body; therefore 20K-hGH may alleviate the hPRLR-mediated side-effects such as breast cancer when administered to human body.

IT Transcription factors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(STAT5; effect of 20K and 22K hGH on cell proliferation, gene promoter activation, and JAK2/Stat5 tyrosine phosphorylation in transfected cells stably expressing human prolactin receptor and human breast cancer cells)

IT Cell proliferation
Signal transduction, biological
(effect of 20K and 22K hGH on cell proliferation, gene promoter activation, and JAK2/Stat5 tyrosine phosphorylation in transfected cells stably expressing human prolactin receptor and human breast cancer cells)

IT Mammary gland
(neoplasm; effect of 20K and 22K hGH on cell proliferation, gene promoter activation, and JAK2/Stat5 tyrosine phosphorylation in transfected cells stably expressing human prolactin receptor and human breast cancer cells)

IT Phosphorylation, biological

(protein; effect of 20K and 22K hGH on cell proliferation,
gene promoter activation, and JAK2/Stat5 tyrosine phosphorylation in
transfected cells stably expressing human prolactin receptor
and human breast cancer cells)

IT 152478-57-4, Jak2 kinase
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL
(Biological study); PROC (Process)
(effect of 20K and 22K hGH on cell proliferation, gene
promoter activation, and JAK2/Stat5 tyrosine phosphorylation in
transfected cells stably expressing human prolactin receptor
and human breast cancer cells)

IT 172522-05-3, Serine protease inhibitor 2.1
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(promoter; effect of 20K and 22K hGH on cell proliferation,
gene promoter activation, and JAK2/Stat5 tyrosine phosphorylation in
transfected cells stably expressing human prolactin receptor
and human breast cancer cells)

L15 ANSWER 6 OF 11 CAPLUS

ACCESSION NUMBER: 1999:403415 CAPLUS
DOCUMENT NUMBER: 131:165530
TITLE: Growth hormone-mediated regulation of insulin-like growth factor I promoter activity in C6 glioma cells
AUTHOR(S): Benbassat, Carlos; Shoba, Lungile N. N.; Newman, Marsha; Adamo, Martin L.; Frank, Stuart J.; Lowe, William L., Jr.
CORPORATE SOURCE: Department of Medicine, Veterans' Affairs Chicago Healthcare System, Lakeside Division, Northwestern University Medical School, Chicago, IL, 60611, USA
SOURCE: Endocrinology (1999), 140(7), 3073-3081
CODEN: ENDOAO; ISSN: 0013-7227
PUBLISHER: Endocrine Society
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 61 THERE ARE 61 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Growth hormone-mediated regulation of insulin-like growth factor I promoter activity in C6 glioma cells

AB The mol. mechanisms by which GH regulates insulin-like growth factor (IGF-I) gene expression remain obscure. One difficulty has been the lack of established GH-responsive cell lines that express the IGF-I gene. To develop such a cell line, the authors used rat C6 glioma cells which, as determined by RNase protection assay, express the IGF-I gene but not the GH receptor gene. To confer GH responsiveness, C6 cells were cotransfected with vectors that express the GH receptor (pRc/CMV WTrGHR) and Jak2 (pRc/CMV Jak2). GH responsiveness was demonstrated using luciferase reporter genes containing either the Sis-inducible element from the c-fos gene (pTK81-SIE-Luc) or 6 copies of the GH-responsive GAS-like element (GLE) from the rat spi2.1 gene (pSpi-GLE-Luc). The SIE is activated by binding of STAT1 and 3, whereas the GLE binds STAT5. In cells cotransfected with pRc/CMV WTrGHR, pRc/CMV Jak2, and either pTK81-SIE-Luc or pSpi GLE-Luc, treatment with 500 ng/mL GH for 24 h stimulated a 3.1- and 1.7-fold increase in luciferase activity, resp. These data suggest that in C6 cells cotransfected with pRc/CMV WTrGHR and pRc/CMV Jak2, GH activates STAT1, 3, and 5. To determine whether GH-responsive IGF-I promoter activity could be demonstrated, C6 cells were cotransfected with pRc/CMV WTrGHR, pRc/CMV Jak2, and an IGF-I-luciferase fusion gene that contained a fragment of the rat IGF-I gene that extended from -412 in the 5'-flanking region of exon 1 to the Met 22 in exon 3. GH stimulated a modest, but reproducible, 1.7-fold increase in luciferase activity in these cells, suggesting that a GH-responsive element is present in this region of the IGF-I gene. To better localize the GH-responsive element, cells were cotransfected with pRc/CMV WTrGHR, pRc/CMV

Jak2 plus one of several IGF-I-luciferase fusion genes containing either fragments of one of the two promoters in the IGF-I gene or a fragment of intron 2 that includes a GH-responsive DNase I hypersensitivity site. For all constructs, treatment with GH for 24 h did not stimulate a significant increase in luciferase activity, suggesting that GH-responsive sequences are not located in these specific regions of the IGF-I gene or that GH-directed transcription of the IGF-I gene is mediated via several different regions of the IGF-I gene and the effect of any one of these regions in isolation was not sufficiently robust to be detected in this model system. In summary, transient expression of the GH receptor and Jak2 in C6 cells creates a GH-responsive system that activates STAT1, 3, and 5. Moreover, a fragment of the IGF-I gene that contains exons 1 and 2, a fragment of exon 3, and introns 1 and 2 is GH responsive using this model system.

IT Animal cell line
(C-6; mol. mechanism for growth hormone regulation of IGF I gene expression in C6 glioma cells)

IT Transcription factors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(STAT1; mol. mechanism for growth hormone regulation of IGF I gene expression in C6 glioma cells)

IT Transcription factors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(STAT3; mol. mechanism for growth hormone regulation of IGF I gene expression in C6 glioma cells)

IT Transcription factors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(STAT5, STAT5a and STAT5b; mol. mechanism for growth hormone regulation of IGF I gene expression in C6 glioma cells)

IT Gene
(expression; mol. mechanism for growth hormone regulation of IGF I gene expression in C6 glioma cells)

IT Signal transduction, biological
Transcriptional regulation
(mol. mechanism for growth hormone regulation of IGF I gene expression in C6 glioma cells)

IT Gene, animal
Growth hormone receptors
Promoter (genetic element)
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(mol. mechanism for growth hormone regulation of IGF I gene expression in C6 glioma cells)

IT 9002-72-6, Growth hormone
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(mol. mechanism for growth hormone regulation of IGF I gene expression in C6 glioma cells)

IT 152478-57-4, Jak2 kinase
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(mol. mechanism for growth hormone regulation of IGF I gene expression in C6 glioma cells)

IT 67763-96-6, Insulin-like growth factor I
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)
(mol. mechanism for growth hormone regulation of IGF I gene expression in C6 glioma cells)

ACCESSION NUMBER: 1998:425181 CAPLUS

DOCUMENT NUMBER: 129:145009
TITLE: Grb10 identified as a potential regulator of growth hormone (GH) signaling by cloning of GH receptor target proteins
AUTHOR(S): Moutoussamy, Soraya; Renaudie, Francoise; Lago, Francisca; Kelly, Paul A.; Finidori, Joelle
CORPORATE SOURCE: Endocrinol. Moleculaire, Fac. Med. Necker-Engants Malades, Inst. National Sante Recherche Med., Paris, 75730, Fr.
SOURCE: Journal of Biological Chemistry (1998), 273(26), 15906-15912
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT
AB The cloning of receptor targets procedure, used so far to identify proteins associated with tyrosine kinase receptors was modified to clone SH2 proteins able to bind to the growth hormone receptor (GHR). The cytoplasmic region of GHR, a member of the cytokine receptor superfamily does not contain tyrosine kinase activity. It was thus phosphorylated in bacteria by the Elk tyrosine kinase and radiolabeled to screen a mouse expression library. With this probe, the authors identified Shc and the p85 subunit of phosphatidylinositol 3-kinase as direct targets of the receptor. The other proteins identified, Csk, Shb, Grb4, and Grb10 are new potential transducers for cytokine receptors. The authors show in Huh-7 hepatoma cells that Grb10 and GHR associate under GH stimulation. Co-transfections in 293 cells further show that Grb10 interacts with both the GHR and Jak2. Functional tests demonstrate that Grb10 inhibits transcription of two reporter genes containing, resp., the serum response element of c-fos and the GH response element 2 of the Spi2.1 gene, whereas it has no effect on a reporter gene containing only Stat5 binding elements. The authors' results suggest that Grb10 is a new target for a member of the cytokine receptor family that down-regulates some GH signaling pathways downstream of Jak2 and independently of Stat5.

L15 ANSWER 8 OF 11 CAPLUS
ACCESSION NUMBER: 1997:127663 CAPLUS
DOCUMENT NUMBER: 126:182229
TITLE: Role of gene overlap in the regulation of mRNA translation for mitochondrial cytochrome P-450c27/25 in the rat
AUTHOR(S): Shayiq, Rass M.
CORPORATE SOURCE: Dep. Pharmacol., Temple Univ. Sch. Med., Philadelphia, PA, 19140, USA
SOURCE: Journal of Biological Chemistry (1997), 272(7), 4050-4057
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Previously published results have revealed sequence complementarity between the 5'-terminal region of mRNAs for hepatic mitochondrial cytochrome P-450c27/25 (c27/25) and serine protease inhibitors (SPI) and predicted a role for this sequence overlap in both the regulation of c27/25 mRNA transcription and translation. The possibility that c27/25 mRNA forms as RNA duplex with complementary sequences of SPI mRNAs in vivo was demonstrated in the rat liver and COS-1 cells cotransfected with c27/25 and SPI2.1 plasmids. Quant. evaluation of RNA duplex in COS-1 cells revealed that most of the c27/25 mRNA exists in duplex form when SPI2.1 mRNA was present at

5-10-fold that of c27/25 mRNA, a ratio comparable to that observed between these two RNAs in the liver. In cotransfected COS-1 cells with the same ratio of mRNAs, highly significant inhibition of the c27/25 mRNA translation (66-75%) was observed, while its transcription remained unaffected. The partial inhibition of c27/25 mRNA translation, even when most of it exists in duplex form, suggests that RNA duplex is undergoing some type of cytoplasmic processing to disengage c27/25 mRNA and make it available for translation. These results imply that abundant endogenous SPI RNAs are able to regulate the c27/25 gene expression.

IT Transformation, genetic
(COS-1 cells cotransfected with c27/25 and SP12.1 plasmids; role of gene overlap in the regulation of mRNA translation for mitochondrial cytochrome P-450c27/25 in the rat)

IT Animal cell line
(COS-1, most of c27/25 mRNA exists in duplex form when SP12.1 mRNA was present at 5-10-fold; role of gene overlap in the regulation of mRNA translation for mitochondrial cytochrome P-450c27/25 in the rat)

IT 139691-92-2, Serine protease inhibitor
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(most of c27/25 mRNA exists in duplex form when SPI2.
1 mRNA was present at 5-10-fold; role of gene overlap in the regulation of mRNA translation for mitochondrial cytochrome P-450c27/25 in the rat)

L15 ANSWER 9 OF 11 CAPLUS

ACCESSION NUMBER: 1997:15306 CAPLUS
DOCUMENT NUMBER: 126:115041

TITLE: Identification of a serpin specifically expressed in multipotent and bipotent hematopoietic progenitor cells and in activated T cells

AUTHOR(S): Hampson, I. N.; Hampson, L.; Pinkoski, M.; Cross, M.; Heyworth, C. M.; Bleackley, R. C.; Atkinson, E.; Dexter, T. M.
CORPORATE SOURCE: CRC Dep. of Experimental Haematology, Paterson Inst. of Cancer Research, Manchester, UK

SOURCE: Blood (1997), 89(1), 108-118
CODEN: BLOOAW; ISSN: 0006-4971
PUBLISHER: Saunders
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Identification of a serpin specifically expressed in multipotent and bipotent hematopoietic progenitor cells and in activated T cells

AB We have identified a gene that has a high level of mRNA expression in undifferentiated, multipotential hematopoietic cells (FDCP-Mix) and that downregulates both transcript and protein, as these cells are induced to differentiate into mature myeloid cells. Sequence anal. of this gene has identified it as a serine protease inhibitor EB22/3 (serpin 2A). Constitutive expression of serpin 2A in FDCP-Mix cells was associated with an increase in the clonogenic potential of the cells and with a delay in the appearance of fully mature cells in cultures undergoing granulocyte macrophage differentiation when compared with control cells. Serpin 2A was also found to be expressed in bone marrow-derived bipotent granulocyte macrophage progenitor cells (GM-colony forming cell [CFC]), but not in erythrocyte progenitor cells from day 15 fetal liver. Expression of serpin 2A also showed a marked up regulation during the activation of cytotoxic suppressor CD8+ T cells, with a clear lag between the appearance of transcript and detection of protein.

ST mouse serpin 2A cloning sequence expression; lymphocyte hematopoietic cell differentiation serpin

2A
IT Animal cell line
(FDCPmix A4; cloning, sequence, and expression of mouse FDCP-Mix cell serpin 2A specifically expressed in multipotent and bipotent hematopoietic progenitor cells and in activated T cells)
IT Hematopoietic precursor cell
Mouse
Protein sequences
cDNA sequences
(cloning, sequence, and expression of mouse FDCP-Mix cell serpin 2A specifically expressed in multipotent and bipotent hematopoietic progenitor cells and in activated T cells)
IT T cell (lymphocyte)
T cell (lymphocyte)
(cytotoxic suppressor cell, activation of, expression of serpin 2A during; cloning, sequence, and expression of mouse FDCP-Mix cell serpin 2A specifically expressed in multipotent and bipotent hematopoietic progenitor cells and in activated T cells)
IT Hematopoietic precursor cell
(granulocyte-macrophage, expression of serpin 2A in; cloning, sequence, and expression of mouse FDCP-Mix cell serpin 2A specifically expressed in multipotent and bipotent hematopoietic progenitor cells and in activated T cells)
IT Cell differentiation
(relationship of to serpin 2A expression; cloning, sequence, and expression of mouse FDCP-Mix cell serpin 2A specifically expressed in multipotent and bipotent hematopoietic progenitor cells and in activated T cells)
IT Animal tissue
(serpin 2A expression in; cloning, sequence, and expression of mouse FDCP-Mix cell serpin 2A specifically expressed in multipotent and bipotent hematopoietic progenitor cells and in activated T cells)
IT Gene, animal
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(serpin 2A, expression of; cloning, sequence, and expression of mouse FDCP-Mix cell serpin 2A specifically expressed in multipotent and bipotent hematopoietic progenitor cells and in activated T cells)
IT 186049-94-5
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(amino acid sequence of; cloning, sequence, and expression of mouse FDCP-Mix cell serpin 2A specifically expressed in multipotent and bipotent hematopoietic progenitor cells and in activated T cells)
IT 186148-63-0, Serpin 2A
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(cloning, sequence, and expression of mouse FDCP-Mix cell serpin 2A specifically expressed in multipotent and bipotent hematopoietic progenitor cells and in activated T cells)
IT 186049-93-4
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(nucleotide sequence of; cloning, sequence, and expression of mouse

FDCP-Mix cell serpin 2A specifically
expressed in multipotent and bipotent hematopoietic progenitor
cells and in activated T cells)

L15 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1992:229465 CAPLUS
DOCUMENT NUMBER: 116:229465
TITLE: Structure and function of a human insulin-like growth factor-I gene promoter
AUTHOR(S): Kim, Sung Woon; Lajara, Rosemarie; Rotwein, Peter
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, MO, 63110, USA
SOURCE: Molecular Endocrinology (1991), 5(12), 1964-72
CODEN: MOENEN; ISSN: 0888-8809
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A promoter regulatory region for the human insulin-like growth factor-I (IGF-I) gene was identified and characterized. The 5'-ends of IGF-I mRNAs were first mapped to a series of sites within a 158-nucleotide portion of exon 1 that was found to be structurally similar to the recently delineated transcription start region of the chicken IGF-I gene. In both species multiple initiation sites are probably a reflection of the absence of typical transcriptional regulatory signals, such as a TATA or CCAAT box, in the proximal promoter, although neither gene sequence resembles a GC-rich housekeeping promoter, which also controls a dispersed initiation region. To test promoter function, hybrid genes were constructed linking human IGF-I DNA to a promoterless reporter plasmid. Fusion genes containing from 385-4300 nucleotides of the IGF-I 5'-flanking region enhanced luciferase activity after transfection into the IGF-I producing SK-N-MC cell line. A plasmid with 1630 nucleotides of 5'-DNA gave maximal activity and directed accurate initiation of the hybrid gene, while the same promoter fragment inserted in the reverse orientation was inert. Although cognate recognition sequences were identified for several transcription factors in the 1630 nucleotides 5' to the transcription start region, no sites were found that resembled the putative GH response element recently mapped to the proximal promoter of the rat Spi2 .1 gene. This study underscores the diversity of mechanisms contributing to IGF-I gene expression by demonstrating that heterogeneous transcription initiation accounts for IGF-I mRNAs with different 5'-ends and provides a starting point for elucidating the ways in which GH and other trophic agents induce IGF-I gene transcription.

L15 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1990:585846 CAPLUS
DOCUMENT NUMBER: 113:185846
TITLE: An inducible nuclear factor binds to a growth hormone-regulated gene
AUTHOR(S): Yoon, Jong Bok; Berry, Susan A.; Seelig, Steven; Towie, Howard C.
CORPORATE SOURCE: Inst. Hum. Genet., Univ. Minnesota, Minneapolis, MN, 55455, USA
SOURCE: Journal of Biological Chemistry (1990), 265(32), 19947-54
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Transcription of the serine protease inhibitor (Spi) 2.1 gene, a member of the serine protease inhibitor family, is induced by growth hormone (GH) in rat liver. To further study the mechanism involved in this process, the Spi 2.1 gene from a rat genomic library was isolated and characterized. Examination of the 5'-flanking region of the Spi 2 gene from normal animals revealed the presence of a DNase I hypersensitive site within 500 bp of the transcriptional initiation site, which was not detectable in hypophysectomized animals. Portions of the 5'-flanking region of the Spi 2.1 gene were fused to a heterologous promoter and reporter gene and introduced into primary rat hepatocytes by lipofection. Spi 2.1 sequences

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      from -275 to -54 gave a 2-3-fold induction of reporter gene activity in
      cells grown in the presence of GH, similar to the level of
      induction of the endogenous Spi 2.1 mRNA in isolated hepatocytes.  Further
      definition of the essential sequences revealed that a segment from -147 to
      -102 could confer GH responsiveness when linked in tandem copies in front
      of a heterologous promoter.  Using the gel shift assay, a nuclear
      factor(s) from normal rat liver was identified which could interact with
      this minimal response fragment.  The importance of this activity to GH
      regulation was suggested by the fact that it was absent in
      hypophysectomized animals but reappeared by 1 h after treatment of such
      animals with GH.  The appearance of this activity was not blocked by
      pretreatment of animals with an inhibitor of protein synthesis, suggesting
      a preexisting factor is modified by GH to yield an activity which
      interacts with the Spi 2.1 gene.
IT    Gene and Genetic element, animal
      RL: BIOL (Biological study)
         (tsp (transcription start point), of serine protease inhibitor gene
         Spi2.1, of rat)


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 NEWS  9  JUN 02  The first reclassification of IPC codes now complete in
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L1           1 WO2001030380/PN
                 (WO2001030380/PN)
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            23 ANTIPROTEINASES
           168 ANTIPROTEINASE
                 (ANTIPROTEINASE OR ANTIPROTEINASES)
L2           0 L1 AND ANTIPROTEINASE

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           153 ?ANTIPROTEINASE
L3           0 L1 AND ?ANTIPROTEINASE

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L3             0 SEA FILE=PCTFULL ABB=ON  PLU=ON  L1 AND ?ANTIPROTEINASE


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         48629 PROTEASE
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L4     ANSWER 1 OF 1      PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:        2001030380 PCTFULL  ED 20020820
TITLE (ENGLISH):         METHODS AND COMPOSITIONS FOR TREATMENT OF KERATOCONUS
                         USING PROTEASE INHIBITORS
TITLE (FRENCH):          TECHNIQUES ET COMPOSITIONS DESTINEES AU TRAITEMENT DU
                         KERATOCONE AU MOYEN D'INHIBITEURS DE PROTEASE
INVENTOR(S):             QUAY, Steven, C.
PATENT ASSIGNEE(S):      K-QUAY ENTERPRISES, LLC;
                         QUAY, Steven, C.
DOCUMENT TYPE:           Patent
PATENT INFORMATION:
                         NUMBER             KIND     DATE
                         --------------------------------
                           WO 2001030380        A2 20010503
DESIGNATED STATES
       W:                AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU
                         CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN
                         IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK
                         MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM
                         TR TT TZ UA UG US UZ VN YU ZA ZW GH GM KE LS MW MZ SD
                         SL SZ TZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY
                         DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG
                         CI CM GA GN GW ML MR NE SN TD TG
APPLICATION INFO.:       WO 2000-US29229      A  20001020
PRIORITY INFO.:          US 1999-60/161,879      19991027
ABEN   Compositions and methods for treating corneal diseases mediated by
       elevated protease activity include ocular administration of
       protease inhibitors. One or more protease inhibitors
       selected from an aspartic, serine, cysteine, or metallo-protease
       inhibitor are administered to an ocular fluid, surface, or tissue,
       preferably by topical administration, to inhibit proteolytic activity
       associated with a corneal disease or condition, for example keratoconus.
       Antiproteolytic formulations of the invention may include carriers that
       prolong the retention and/or enhance delivery of the protease
       inhibitor. These formulations can also include other therapeutic agents
       such as antiinflammatory or antibiotic drugs.. In preferred aspects of
       the invention, antiproteolytic formulations are administered during
       periods of closed eye tear production. Also provided within the
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invention are implant devices for corneal delivery of a protease inhibitor.

ABFR

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L5            1 L4 AND ANTI?

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L5 ANSWER 1 OF 1 PCTFULL

TIEN METHODS AND COMPOSITIONS FOR TREATMENT OF KERATOCONUS USING PROTEASE INHIBITORS

TIFR TECHNIQUES ET COMPOSITIONS DESTINEES AU TRAITEMENT DU KERATOCONE AU MOYEN D'INHIBITEURS DE PROTEASE

PI WO 2001030380 A2 20010503

ABEN Compositions and methods for treating corneal diseases mediated by elevated protease activity include ocular administration of protease inhibitors. One or more protease inhibitors selected from an aspartic, serine, cysteine, or metallo-protease inhibitor are administered to an ocular fluid, surface, or tissue, preferably by topical administration, to inhibit proteolytic activity associated with a corneal disease or condition, for example keratoconus. Antiproteolytic formulations of the invention may include carriers that prolong the retention and/or enhance delivery of the protease inhibitor. These formulations can also include other therapeutic agents such as antiinflammatory or antibiotic drugs. In preferred aspects of the invention, antiproteolytic formulations are administered during periods of closed eye tear production. Also provided within the invention are implant devices for corneal delivery of a protease inhibitor.

DETD METHODS AND COMPOSITIONS FOR TREATMENT OF KERATOCONUS USING PROTEASE INHIBITORS

BACKGROUND OF THE INVENTION

Keratoconus is a bilateral ocular disorder that progressively thins and distorts the central portion of the cornea toward. . .

107:929-936, 1982, incorporated herein by reference. In light of these reports, some investigators propose that imbalances in protease/ protease inhibitor levels may contribute to decreased protein levels and increased proteolytic activities associated with keratoconus. Additional investigations have reported decreases in the levels of certain proteolytic enzyme inhibitors, for example, a I -protease inhibitor (alp 1) (Whitelock et al., Invest. Oplithalmol. Vis. Sci. 38:529-534, 1997; Sawaguchi et al., En. Eye Res. 50:549-5545 1990, each. . .

However, the model of a protease/protease inhibitor imbalance as a pathogenic mechanism in keratoconus remains speculative. In this regard it is noteworthy that Yue et al., supra, conclude. . . cases where collagen levels are actually reduced, the reduction is apparently due to decreased collagen synthesis rather than to an increase in protease activity or a decrease in protease inhibition. Other reports suggest that pathogenic changes associated with keratoconus, including increased collagenolytic activity, may be attributed to

structural changes in the collagen proteins themselves, as opposed to altered collagen expression or elevated protease ftmction (e.g., attributable to higher protease levels or activities, or decreased protease inhibitor levels or activities).

Data obtained by assaying acyl transferase activity show that MMPs account for at least 95% of the total protease secreted by cultured keratocytes. The summated specific activity of MMI's is reported to be consistently and significantly higher in the culture media. . .

. . .

and casein are preferred substrates for gelatinases A and B, and stromelysin. They can, however, also serve as substrates for other proteases.

To determine whether gelatinolytic and caseinolytic activities associated with keratoconus are caused by MMPs or other classes of proteases, Zhou and coworkers (1998, supra) also employed inhibitors specific for four classes of proteases (aspartic, serine, cysteine, and metallo-proteases) as blocking reagents. These studies reportedly showed that, in both healthy controls and keratoconic specimens, the net gelatinolytic and caseinolytic activities were related mostly to serine and cysteine proteases, and not to aspartic proteases, gelatinases A and B, or stromelysin. The inhibitor of serine proteases phenylmethyl sulfonyl fluoride, the cysteine protease inhibitor E-64, and the cathepsin 13-trypsin inhibitor leupeptin substantially reduced digestion of gelatin and casein, whereas the aspartic protease inhibitor pepstatin and the MMP inhibitor 1, I 0-phenanthroline failed to block the reaction.

. . .

Zhou and coworkers suggest that most gelatinolytic and caseinolytic activities in healthy human comeas may not be caused by MMPs. Instead, cysteine proteases, such as cathepsin B, and serine proteases, such as cathepsin G, both of which are reportedly elevated in keratoconus, are proposed to contribute to the enhanced gelatin- and casein-digesting. . .

Chem. 267:21830-21838, 1992, incorporated herein by reference). Evidence presented in these and other reports suggests that certain protease inhibitors are reduced in association with keratoconic disease. For example, a decrease in al-protease inhibitor levels were reported in both the epithelial and the stromal layers of keratoconus comeas based on immunostaining and dot blot assays.. . . inhibitor level actually occurred in the keratoconus strornal cells. Other evidence based on measurements of rnRNA levels suggest that the a- I -protease inhibitor may be downregulated in association with keratoconus. Whitelock et al. (Invest. Opthalmol. Vis. Sci. 38:529-534, 1997, incorporated herein by reference).

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Despite the uncertainties apparent from these reports, a number of patent disclosures purport to teach methods for treating corneal disease using protease inhibitors.

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Levy et al., and U.S. Patent No. 5,892,112, issued April 6, 1999 to Levy et al., state that synthetic, small molecule, non-peptide protease inhibitors may be useful in treating keratoconus. However, these disclosures add nothing to the foregoing reports with respect to identifying the. . . potential treatment agents and modalities for clinical use against the disease. Moreover, each of these disclosures proposes that vast numbers of synthetic protease inhibitors may be used to treat a laundry list of diseases.

Thus, both of the Levin et al. patents identify hundreds of synthetic protease inhibitors and generally assert that the disclosed compounds can be used to treat such diverse diseases and conditions as arthritis, tumor growth. . .

Most notably, none of the foregoing patents directed toward production and use of small molecule protease inhibitors for disease treatment provide specific direction or guidance as to the underlying mechanisms of keratoconus and other corneal diseases, nor. . .

Further discussion regarding the possible roles of proteases and their inhibitors in keratocomis is also provided by Kenney et al., Biochem. Biophys. Res.

Based on these results, Kenney et al. propose an alternative mechanism to the protease/protease inhibitor imbalance model proposed by others. In particular, the authors suggest that there may be an inherent difference between the diseased. . .

Reported changes in protease and protease inhibitor properties (e.g., expression, structure, activity or metabolism) are difficult to interpret, and to reconcile with other reports.

. . .

of keratoconus, or merely represent incidental sequelae of the disease. Resolution of these alternative possibilities is further complicated by the possibility that protease and protease inhibitor activities may be altered in keratoconus as a result of concurrent wound-healing and repair mechanisms, allergic responses, or responses to mechanical trauma, rather than as a direct effect of the primary disease. For example, declines in the protease inhibitor levels in the cornea may simply be a reflection of a similar decline in tears, or in serum.

. . .

Endocrinology 108:2129-2135, 1981) and allergic disease (Berman et al., Invest. Opthalmol. Vis. Sci. 12:759-770, 1973,

each incorporated herein by reference) can affect protease inhibitor levels in tears and in serum, and allergic diseases are often associated with keratoconus (Rahi et al., J.

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may be influenced by a variety of complex molecular and biochemical factors. In this context, recent discussion has been directed toward specific proteases as potential modulators of proteoglycan structure/metabolism. Cathepsin B, a cysteine protease, and cathepsin G, a neutral serine protease, are both enzymes known to degrade proteoglycans in the corneal stroma, which is the site of thinning and scarring in keratoconus..

The methods of the invention involve ocular administration of an antiproteolytic effective amount of a protease inhibitor in an opthalmically acceptable carrier. The protease inhibitor is preferably a protein or peptide protease inhibitor selected from an aspartic, serine, cysteine, or metallo-protease inhibitor, which may be derived from a natural source or produced in a native or modified form by recombinant or synthetic techniques known in the art. In most instances, recombinant or synthetic protease inhibitors are preferred, as these materials will generally be free of undesirable contaminants and infectious agents.

According to the methods of the invention, a protease inhibitor formulation is administered to an ocular fluid, surface, or tissue, preferably by topical administration, in an antiproteolytic effective amount to substantially inhibit a proteolytic activity associated with the corneal disease or condition to be treated. Proteolytic activities in this context may include activities of multispecific or specific proteases, complex formation between a protease and protease inhibitor, histopathological changes in the cornea attributed to proteolytic processes, and other indicia correlated with proteolytic mechanisms.

The antiproteolytic formulations of the invention can include various carriers that prolong retention and/or enhance delivery of the inhibitor. Optionally, the antiproteolytic formulations can include permeabilizing agents and preservatives, which may be a single agent that enhances permeability and provides a simultaneous preservative function. In addition, the formulation can include a plurality of protease inhibitors, as well as other therapeutic agents such as antfinflaminatory or antibiotic drugs.

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to the methods of the invention vary in accordance with a variety of factors detailed below. In preferred

aspects of the invention, antiproteolytic formulations are administered during periods when contact lens are wom and/or of closed eye tear production to enhance therapeutic efficacy.

Also provided within the invention are implant devices adapted for corneal delivery of an effective amount of an antiprotease. These devices are provided in the

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form of an ocular implant having a concave inner surface similar in size and shape. . . . cornea of a patient suffering from a corneal disease or condition and serves as a carrier to deliver to the cornea an antiproteolytically effective amount of a protease inhibitor.

. . . .

and compositions for treating or preventing corneal disease in a mammalian patient. The methods of the invention involve ocular administration of an antiproteolytic effective amount of a protease inhibitor in an opthalmically acceptable carrier. The protease inhibitor is preferably selected from an aspartic, serine, cysteine, or metallo-protease inhibitor, obtained from a natural source or produced in a native (i.e., wild-type amino acid sequence) or modified (e.g., by amino acid. . .

According to the methods of the invention, a protease inhibitor formulation is administered to an ocular fluid, surface, or tissue, preferably by topical administration, in an antiproteolytic amount effective to substantially inhibit a proteolytic activity associated with the corneal disease or condition to be treated.

By substantial inhibition of proteolytic activity is meant that administration of the protease inhibitor formulation yields at least about a IO% reduction of proteolytic activity, preferably at least a 20% reduction, compared to a. . . . target site, for example within the extracomeal fluid, corneal epithelium, corneal stroma, Bowman's layer, or the vitreous humor. Preferably, administration of the protease inhibitor yields approximately a 30-50% reduction of proteolytic activity, more preferably greater than about a 50% reduction, and in some preferred aspects. . .

As used herein, proteolytic activity refers to a quantitative digestive activity of a target protease against a protein (e.g., collagen, elastin, fibronectin) or glycoprotein (e.g., a proteoglycan or glycosaminoglycan) substrate. Target proteases as herein defined include proteolytic enzymes that exhibit aberrantly high levels of expression or activity (e.g., attributable to structural changes that increase substrate

1 8

binding or otherwise enhance digestion kinetics, or that render the protease more susceptible to activation from a proenzyme to an active form), or whose

regulation (e.g., by metabolic turnover, protease inhibition or other mechanisms) is impaired in association with a corneal disease or condition to be treated, for example keratoconus or corneal infections. As further defined herein, target proteases are amenable to regulatory inhibition by exogenously administered protease inhibitors.

Proteases that may be successfully targeted for inhibition by the compositions and methods of the invention include, but are not limited to,. . .

Antiproteolytic activity may be determined by, e.g., various quantitative, in vitro or in vivo assays, for example by enzymatic and/or immunological assays. . . samples, samples from keratocyte culture media, or corneal tissue samples, as described herein below and as otherwise known in the art. Alternatively, antiproteolytic activity may be determined by other indicia, for example by quantitative changes in morphological or ultrastructural features attributable to proteolytic activity. . .

The extent of antiproteolytic activity elicited by the compositions and methods of the invention (i.e., for determining efficacy and calibrating dosages) can be deten-nined by a. . . in the examples below. Suitable in vitro test and control samples include cultured, normal and keratoconic keratocytes, respectively, each treated with an antiprotease formulation of the invention. Using these samples, protease inhibition can be measured at selected time points, for example, by assaying target protease-inhibitor complex formation, rates or levels of protein digestion attributed to the target protease,
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morphological indicia as noted above, and other parameters consistent with the quantitative values sought.

. . . with keratoconic and normal corneas to provide, respectively, test and control samples for in vitro assays. For quantitative determination of in vivo protease inhibition, test and control samples may include extracorneal fluid or corneal tissue samples taken from subjects (e.g., a human or non-human mammal such as a rabbit) following administration of a protease inhibitor formulation (test sample), and following administration of a placebo comprising, e.g., a selected carrier without the protease inhibitor (control sample). Often, test and control samples will be provided by bilateral administration of test and control treatments to an individual. . .

Protease inhibitors that are useful within the invention are any of the inhibitors, their analogs, recombinantly modified variants,

proteolytically active fragments, derivatives, or salts, which can inhibit tarizet proteases as defined above.

herein that provide for enhanced absorption, retention and delivery of the inhibitor at a site of treatment. In various preferred embodiments, the protease inhibitor may be selected from an aspartic, serine, cysteine, or metallo-protease inhibitor. Useful inhibitors may be derived from a natural source or produced in a native or modified fonn by recombinant or synthetic techniques known in the art. In more detailed aspects of the invention, protease inhibitors bind with one or more proteases that exhibit increased levels of expression or activity, or aberrant regulation, leading to pathogenic protein or glycoprotein degradation and/or morphologic changes associated with. . .

As noted above, preferred protease inhibitors include native or modified aspartic, serine, cysteine, or metallo-protease inhibitors. Exemplary inhibitors in this context include al-antiprotease (alpl, formerly known as al-antitrypsin), a2-macroglobulin (a2-M), secretory leucocyte protease inhibitor (SLP I, formerly known as mucus proteinase inhibitor and antileukoprotease), 0 1 - antigellagenase, a2-antiplasmin, serine amylyoid A protein, al -antichymotrypsin (al -Achy), cystatin C, inter-a-trypsin inhibitor, elafin, elastinal, aprotinin, phenylmethyl sulfonyl fluoride, the cysteine protease inhibitor E-64, the eathepsin B-trypsin inhibitor leupeptin, and the metalloprotease inhibitors TIMP-1, TIMP-2, and 1, 10-phenanthroline.

A particularly preferred protease inhibitor for use within the compositions and methods of the invention is a2-maeroglobulin. This inhibitor is a high-molecular-weight (718 W), homotetrameric glycoprotein implicated as a regulator of degradation for certain extraeellular matrix components and other macromolecules. Unlike many other protease inhibitors, a2-maeroglobulin is not highly specific for a preferred target protease, and is not particularly fast acting. Instead, a2-macroglobulin inhibits proteases from all four major classes and is considered to be relatively slow in its activity.

Consistent with these properties, the mechanism of action by a2-macroglobulin is also unique. When this protease inhibitor reacts with a target protease, proteolytie cleavage in the bait region of the inhibitor occurs, leading to a eonformational change and trapping of the protease. A covalent bond is then formed between the protease and a2-macroglobulin. The protease-inhibitor complex is ultimately cleared from the circulation

1 5 by a receptor-mediated mechanism.

Another preferred protease inhibitor for use within the compositions and methods of the invention is a-1 protease inhibitor (alpl), a major protease inhibitor in human plasma synthesized mainly by parenchymal liver cells. Alp I is a glycoprotein of 53 kDa that forms a 1:. . .

As noted above, useful compositions within the invention include forinulations of antiprotease salts, derivatives and complexes. As used herein, the term pharmaceutically acceptable salts, derivatives and complexes retain the desired biological activity of the corresponding native antiprotease, and exhibit minimal undesired toxico- logical effects. Nonlimiting examples of useful antiprotease salts are acid addition salts formed with inorganic acids (for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and. . .

Pharmaceutically acceptable derivatives and complexes of protease inhibitors include native or modified inhibitors that are chemically modified (e.g., by addition of stabilizing or otherwise functional chemical moieties), truncated, conjugated (e.g.,. . . cDNA encoding the inhibitor to introduce substitute, or delete non-critical amino acid residues), which retain desired biological activity of the corresponding native antiprotease.

Particularly useful in this context are protease inhibitor analogs, which comprise recombinantly modified variants and proteolytically active fragments of native inhibitors. These analogs preferably exhibit at least 80% amino. . .

Antiprotease analogs preferably share substantial amino acid sequence identity (e.g., at least 75%, preferably 80%, and more preferably 95% or greater sequence identity). . .

For practicing the methods of the invention, the precise amounts of protease inhibitors to be administered and the frequency and duration of treatment will depend on the status of the corneal condition or. . . will vary such that specific regimens can be established by those skilled in the art to maximize efficacy of treatment. Ordinarily, the antiprotease is administered in a dosage of between approximately 0.2]tg/ml and 1.0

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mg/ml. Preferably,theinhibitorispresentinaconcentrationofaboutO I.Ogg/ml, more preferably at a concentration of about 0.5 gg/ml. Exemplary formulations for a I - antitrypsin will comprise the inhibitor at approximately the same range of concentrations, with the most preferred concentration being between about 1.0 and. . .

In a preferred aspect of the invention, compositions comprising a protease

inhibitor are administered during periods of closed eye tear production (e.g., during a patient's sleep periods). This method greatly enhances antiproteolytic results, yielding prolonged inhibition of proteolytic processes in corneal tissues (e.g., as demonstrated by reduction in the activity of specific target protease(s)), and long-term inhibition of histopathological changes, such as fragmentation of Bowman's layer. Administration of the antiprotease compositions of the invention during periods of closed eye tear production greatly enhances the antiproteolytic efficacy of these compositions compared to the efficacy achieved by antiprotease administration during periods of reflex tear production, although the latter use is effective and within the scope of the invention. This is due in part to the prolonged retention of the antiprotease composition attributable to a reduction in tear flushing between closed eye and reflex tear periods. This enhanced efficacy is also attributable to fundamental differences in the processes and regulation of proteolysis that characterize the closed eye, versus reflex tear environments. The protease inhibitor compositions and pharmaceutical formulations of the invention can also be administered during a period that is concurrent with or closely preceding a medical procedure or other event anticipated to produce a risk of proteolytic injury, for example following eye surgery or during bacterial infection. Thus, methods are provided which involve administration of an antiproteolytic composition concurrent with, or within an antiproteolytic effective period preceding or following, a surgical procedure or infection, whereby the administration reduces or eliminates risk of deleterious proteolytic responses normally. . .

Within the methods of the invention, formulations comprising a protease inhibitor, a mixture of a plurality of protease inhibitors, or a mixture of one or more protease inhibitors combined with a second therapeutic agent (e.g., an antibiotic, antiviral or antiinflammatory drug) can be administered by a variety of routes, including via topical administration (using, e.g., drops, gels, creams or microparticles as. . .

Preferred methods of the invention involve coordinate (e.g., simultaneous or closely contemporaneous to yield coordinate treatment) administration of a plurality of antiprotease proteins, analogs, salts, or derivatives, or administration of formulations comprising multiple protease inhibitors that may be admixed or complexed. Practice of these methods reduces abnormal proteolytic mechanisms attending a targeted corneal disorder (e.g., keratoconus) at combinatorial antiproteolytic

levels that exceed antiproteolytic levels observed when either of the coordinately administered protease inhibitors are administered alone. This inhibition, as when other compositions and methods of the invention are employed, reduces proteolytic activity in extracomeal fluid (tears), and in corneal tissues (as determined by both enzymatic and histopathological assays). In preferred embodiments, a multispecific protease inhibitor (i.e., an inhibitor which targets multiple protease species), such as a2-M, SLP I and alp 1, is coordinately administered with another multispecific inhibitor, or, alternatively, a multispecific inhibitor is coordinately administered with an oligospecific or specific inhibitor (the latter types of inhibitors represented, e.g., by 0 1 -antigellagenase , a2-antiplasmin, serine amylyoid A protein, al-antichymotrypsin (al-Achy), cystatin C, inter-a-trypsin inhibitor, elafin, elastinal, aprotinin, phenylmethyl sulfonyl fluoride, leupeptin, and the metalloprotease inhibitors TIMP- 1, TIMP-2, and 1, I 0-phenanthroline). Using these combinatorial compositions and treatment methods, the invention achieves effective inhibition against multiple proteases (and/or their pathogenic effects) involved in a particular corneal disease process. Thus, the methods and compositions of the invention provide antiproteolytic effects against a broad range of proteases, including but not limited to, acid esterases, acid phosphatases, acid lipases, cathepsins, collagenases, elastases, tryptases, chymases, kinins, kalikreins, tumor necrosis factors, chymotrypsins,
26
stromelysins,. . .

Additional preferred methods of the invention involve coordinate administration of an antiprotease and an antibiotic, or administration of fon-nulations comprising both a protease inhibitor and an antibiotic . Practice of these methods reduces abnormal proteolytic mechanisms attending a targeted corneal disorder (e.g., keratoconus) and also secondarily reduces proteolytic effects attributed to bacterial infection. Useful antibiotics may be any opthalmically acceptable antibiotic indicated for treatment of an ocular bacterial infection, including, but not limited to fluoroquinolones (e.g., ofloxacin, norfloxacin, ciprofloxacin), gentamicin, and pilocarpine.

Other medications useful in these combinatorial treatment methods include steroidal antiffiflarnmatories, such as corticosteroids, and nonsteroidal antiinflammatories, for example aspirin, ibuprofen, indomethacin, fenoprofen, mefenamic acid, flufenarnic acid, and sulindac. Many other combinatorially effective medicaments useful for
1 5 coordinate ophthalmic. . .

Typically, the protease inhibitors, analogs, salts, derivatives, and coordinately administered therapeutic agents of the invention will be administered in the form of a pharmaceutical composition,. . .

In other embodiments of the invention, the protease inhibitors, analogs, salts, derivatives, and coordinately administered therapeutic agents of the invention are prepared with carriers that protect the compound against rapid. . . .

novel delivery device is provided in the form of an ocular implant adapted for corneal delivery of an effective amount of an antiprotease. The device has a concave inner surface that conforms to an external surface of the cornea, i.e., which is similar in. . . of a patient suffering from a corneal disease or condition. The device serves as a carrier to deliver to the cornea an antiproteolytically effective amount of a protease inhibitor. Preferably, the device is comprised of a gas-permeable, biocompatible polymer, such as ethylene vinyl acetate, polyanhydride, polyglycolic acid, collagen, polyorthoester, or polylactic acid. The entire body, or at least an inner surface, of the device is coated or impregnated with the protease inhibitor. The device is disposable and provided in sterile packaging, to be implanted by the patient and worn for a selected treatment. . .

Liposomal suspensions may provide useful, pharmaceutically acceptable carriers for formulating antiproteolytic compositions of the invention. These may be prepared according to methods known to those skilled in the art, for example, as described. . . is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the protease inhibitor is then introduced into the container. The container is swirled by hand to free lipid material from the sides of. . . .

tonicity-adjusting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, etc. The concentration of the antiprotease in these formulations can vary widely, i.e., from less than about 0.05%, usually at least about 0.5%, to as much as 15. . .

In preferred methods within the invention, mammalian subjects, including human patients, suffering from protease-mediated corneal disorders are treated by administering to the patient a pharmaceutical or therapeutic composition comprising an effective amount of one or more antiproteases, or a pharmaceutically acceptable derivative or complex thereof, in a pharmaceutically acceptable carrier or diluent.

The active antiprotease is included in the pharmaceutically

acceptable carrier or diluent in an amount sufficient to deliver to a patient an antiinflammatory effective amount without causing serious adverse side-effects in the patient treated. The active compound is preferably administered to achieve peak concentration of the antiprotease in tear fluid or corneal tissue of the patient within about 1-4 hours after administration. Concentration of the antiprotease in pharmaceutical compositions and devices of the invention will depend on such factors as absorption, distribution, inactivation, degradation, and flushing of the antiprotease, as well as other factors known to those of skill in the art. It is to be noted that dosage values. . .

. . .

wherein EC50 is the concentration of compound that provides 50%

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inhibition of a target proteolytic activity (e.g., proteolysis by a specific protease, or histopathologic change attributed to proteolysis) compared to a relevant control, and IC50 is the concentration of compound that is toxic to. . .

EXAMPLE I

PROTEASE INHIBITION AND DETERMINATION OF ANTIPROTEOLYTIC ACTIVITY IN TEAR SAMPLES

The present example describes representative protocols for determining inhibition of proteolytic activity in extracorneal fluid (tears) of rabbits and human subjects treated using the compositions and methods of the invention. This example involves ocular administration of one or more protease inhibitors to suitable test and control subjects, followed by immunological and/or enzymatic assays to identify and quantify resultant proteolytic activity and relevant. . . the following protocols, which exemplify suitable assays for determining caseinolytic activity, are adaptable for additional proteolysis assays using various sample types, target proteases, and protease inhibitor types, concentrations, and formulations (e.g., eyedrops, topical gels, microparticulate solutions, etc.). In particular, the methods described herein below are readily adapted to further define preferred compositions and methods of the invention by determining, e.g., (1) optimal timing and duration of protease inhibitor treatments; (2) optimal inhibitor type(s) for inhibiting different target protease(s) (e.g., by selecting different classes or species of preferred inhibitor(s), or by using inhibitor variants such as recombinant or synthetically modified inhibitors);. . .

In the case of in vitro and in vivo antiproteolytic assays involving non-invasive collection methods, e.g., collection of tear samples and postoperative or postmortem collection of tissue samples, human subjects are. . .

. . .

in the following examples are conducted using samples

taken from test and control subjects, typically before, during, and after treatment with a protease inhibitor formulation. In general, these assays will incorporate standard proteolytic activity assays along with control assays that determine relevant associated parameters, for example, levels of target proteases and their substrate proteins, quantitative measurements of proteolytic activity (e.g., based on detection of substrate

3 1

protein levels, inhibitor cleavage products, protease /inhibitor complexes, or based on enzymatic assay readings), and levels of endogenous and exogenous protease inhibitors.

The mode, timing, and duration of treatment is also varied as described in further detail below. Most commonly, antiproteolytic formulations of the invention are administered topically, e.g., in the form of drops, gels, creams, microparticulate solutions, and the like. Alternatively, antiproteolytic formulations are delivered directly to a corneal tissue or other site (e.g., vitreous humor) using, e.g., permeabilizing formulations or suitable injection methods.

For determining short-term delivery kinetics and efficacy, antiproteolytic formulations are administered to subjects in a one-time dose, and proteolytic activity and associated parameters are determined for samples taken. . . (e.g., at I hr, 4 hr, 8 hr, 16 hr, and 24 hr post-administration). For determining longer-term delivery kinetics and efficacy, antiproteolytic formulations are administered to subjects in a single, sustained-delivery dose, or in multiple doses (e.g., daily), and measurements of proteolytic activity and. . .

Concentration of protease inhibitors and other agents incorporated within the formulations of the invention will vary in accordance with such factors as the delivery vehicle. . .

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a concentration of about 0 1.0]tg/ml, more preferably at a concentration of about 0.5 gg/ml. Exemplary formulations for a I - antitrypsin will comprise the inhibitor at approximately the same range of concentrations, with the most preferred concentration being between about 1.0 and. . .

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(pre-conditioned with HPLC buffer) for further analysis. To confirm the relative size range of the eluted material, the column is calibrated, using protease and antiprotease standards and a commercial mixture of gel filtration chromatography standards (Bio-Rad).

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Eye. Res. 16:810-819, 1997, incorporated herein by reference). The methods set forth below are described for caseinolytic assays detecting and/or quantifying selected proteases (elastase, cathepsin G and proteinase-3). protease

inhibitors (alp 1, a 1 -Achy, SLP 1, elafin, and a2-M), and protease/inhibitor complexes.

However, these methods are also routinely adaptable for detecting and measuring other useful proteases and protease inhibitors within the invention, e.g., by substituting the specific immunological probes, enzymatic reagents, etc.

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per gel, until the lowest molecular weight marker (See Blue TM markers, Novex, San Diego, CA) reaches the bottom of the gel. Protease standards, for example human glu-plasmin (American Diagnostics Inc., Greenwich, CT), cathepsin G, elastase (Calbiochem, La Jolla, CA) and proteinase-3 (Elastin product Company,. . .

. . . .

are selected to overcome these problems so as to provide useful quantitative data, as demonstrated in the above cited reports for the protease inhibitors a2-M, alpl (5 8 kDa), a 1 -Achy (6 8 kDa), SLPI (I 1.7 kDa), elafin (6-9 kDa), cystatin C. . .

. . . .

assay, the conditions of electrophoresis (such as mode of sample preparation, types and percentage of gels) are optimized to obtain maximal antigenic 0 sensitivity and to resolve the antigens in question from overlapping blocking tear proteins as well as from non-specific reacting sub-stances. High and mid-molecular weight range antigens are separated by fine tuning the conditions of separation on pre-cast 4-20% gradient minigels. Low molecular weight entities are similarly separated. . .

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IgG1 (Jackson Immunochemicals, West Grove, PA and Sigma, St. Louis, MO), which are run, processed and analyzed simultaneously. Exemplary positive controls for protease inhibitors include elafin (Peptides International, Louisville, KY), SLPI (R and D System Inc., Minneapolis, MN), a I -Achy, alpl, a2-M and cystatin. . .

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20% v/v methanol, 25 mM Tris-Base, 192 mM Glycine buffer pH 8.3 and transferred (Bio-Rad miniblot apparatus) overnight at 30V at 4'C. Antigens larger than 14 kD are transferred onto Immobilon-P membranes, those
35
smaller than 14 kDa are transferred onto Iminobilon-PSq (pore size 0..

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block (Tropix, Bedford, MA)). This is followed by incubation for I h in 1: I 00- 1:2,000 v/v dilution of primary antibody in blocking buffer.

To maximize detection of reactive products and complexes, anti -bodies with known narrow target antigens are avoided. In this context, exemplary primary antibodies for detecting proteases and protease inhibitors are

sheep polyclonal antibodies to elastase, a2-M and alpl (Binding Site), rabbit polyclonal antibodies to cystatin C (DAKO, Glostrup, DK), proteinase-3 (Elastin product company), elafin (Peptides International) and cathepsin G (Calbiochem), goat polyclonal antibodies to SLPI (R and D systems, Inc.) and monoclonal antibodies to a 1 -Achy (Calbiochem).

After primary antibody incubation and washing, the membranes are incubated for 1 h in a 1:30,000 v/v dilution of an appropriate alkaline-phosphatase conjugated secondary antibody affinity purified against human serum proteins (Sigma), in blocking buffer. The membranes are then washed and reacted with NBT-BCIP substrate (Pierce, Chicago,. . .

To estimate concentrations of inhibitor-protease complexes and degradative products, all detected species are assumed to exhibit antigenicity similar to the native antiprotease. Although it is recognized that this will not always hold true, loss of detected epitopes between free and complexed species will. . .

Conventional Western blot procedures are too insensitive to allow detection of nanogram quantities of polymorphonuclear leucocyte (PMN) cell proteases.

Accordingly, an alternate blotting protocol is employed involving pre-incubation of the samples with detergent and a protease inhibitor, electrophoresis at 4'C (to reduce adherence and proteolysis), and blot transfer under basic conditions (to enhance the
36
efficiency of transfer of. . .

Immunoprecipitation Reactions
C tears and the HPLC fractions are immunoprecipitated with antibodies to a selected protein, glycoprotein, protease or antiprotease by incubation at VC overnight.

By applying the foregoing procedures and principles, it will be shown that the antiproteolytic compositions and methods of the invention effectively inhibit proteolytic activity in extracomeal fluid (tears). By selecting a multispecific protease inhibitor (i.e., an inhibitor which targets multiple protease species) such as a2-M and alp 1, or by combining multiple protease inhibitors in a single treatment formulation, effective inhibition can be achieved against multiple proteases involved in a corneal disease process. Accordingly, the methods of the invention will be shown to inhibit corneal disease mediated by exposure of the cornea to aberrantly elevated protease activities in tears.

Adaptation of the foregoing procedures as disclosed herein will further demonstrate that antiproteolytic effects against a broad range

of proteases (e.g., acid esterases, acid phosphatases, acid lipases; cathepsins, collagenases, elastases, tryptases, chyrnases, kinins, kalikreins, tumor necrosis factors, chymotrypsins, stromelysins, and matrix metalloproteases) that alleviate or prevent corneal disease can be achieved by ocular administration of protease inhibitors selected from a broad range of inhibitors, including aspartic, serine, cysteine, or metallo-protease inhibitors. These may be obtained from natural or artificial sources, and can include modified inhibitors that vary in structure from native inhibitors while retaining substantial antiprotease activity.

Species of protease inhibitors useful within this context include alp 1, a2-M, SLP 1, 0 1 -

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antigellagenase, a2-antiplasmin, serine amylyoid A protein, a 1 -Achy, cystatin C, inter-a-trypsin inhibitor, elafin, elastinal, aprotinin, phenylmethyl sulfonyl fluoride, E-64, leupeptin, and the. . .

Quantitative analyses based on the foregoing procedures will further demonstrate that administration of the antiproteolytic compositions of the invention yields at least about a 20% reduction of proteolytic activity compared to a relevant baseline or control value. . . .

EXAMPLE 11

1 5 ENHANCED PROTEASE INHIBITION IN CLOSED-EYE TEARS AND ASSOCIATED INHIBITION OF PROTEOLYSIS AND ASSOCIATED HISTOPATHOLOGIC CHANGES IN KERATOCONIC CORNEAS

The present example describes representative protocols for. . . in the cornea of patients suffering from keratoconus. As in the preceding example, this protocol involves ocular administration of one or more protease inhibitors to suitable test and control subjects, followed by immunological and/or enzymatic assays to identify and quantify resultant proteolytic activity and associated. . .

However, the present example is specifically directed to administration of an antiprotease composition to patients during periods of closed eye tear (C tear) production. In addition, the protocol of the instant example incorporates. . . and/or histopathological analyses of corneal tissues, in addition to the above described assays using tear samples. These additional assays allow confirmation that protease inhibition in closed eye tears results in associated inhibition of proteolytic processes in corneal tissues (e.g., as demonstrated by reduction in the activity of specific target protease(s), or by long-term inhibition of histopathological changes such as fragmentation of Bowman's layer).

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Administration of the antiprotease compositions of the invention during

periods or closed eye tear production greatly enhances the efficacy of corneal therapy
according to the methods of the invention. This is due in part to the prolonged retention
of the antiprotease composition attributable to a reduction in tear flushing between closed
eye and reflex tear periods. This enhanced efficacy is also attributable. . .
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mediators from the ocular
surfaces. Concordant with this capability, the open eye fluid is of limited nutritional
value and is enriched in anti-microbial and antiinflammatory constituents (van Haeringen,
Sury. Ophthalmol. 26:84-96,1981; KijIstra, Rep. Immunol. 3:193-197,1991, each
incorporated herein by reference).

It has been shown that the anti-inflammatory armament of reflex tears
includes trace amounts of several serpins: alpl, al-Achy and a2-M, whereas the principal
serpin in R tears is. . . by
reference. Based upon the calculated molar ratios of the observed serpins and the unique
presence of two rather than one serine protease binding sites on SLPI, Sathe and
coworkers report that SLPI accounts for 95% or more of the total elastase inhibitory
activity in. . .
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SLPI and alp I
function in a synergistic manner in other mucosal tissue to neutralize the entire spectrum
39
of PMN cell serine proteases (Bergenfeldt et al., Scandinavian Journal of Clinical &
Laboratogy Investigatio 52:823-829, 1992, incorporated herein by reference).

SLPI has antimicrobial properties that are consistent with a barrier defense
function. SLPI is cytotoxic to E. coli and Staphylococcus aureus (Hiemstra et al.,
Infection. . .

While the serine protease neutralizing capacity of R fluid is limited, in the
open eye during inflammation or infection this capacity can be readily augmented
through: (1) an increased rate of fluid turnover, (2) an increased inclusion through
vascular leakage of mid-molecular weight range serum antiproteases (e.g. alp I) and (3y a
possible up-regulation of locally synthesized antiproteases. Upon eye closure the
situation changes dramatically.
.
Ophthalmol. Vis. Sci. 33:626-640, 1992, incorporated herein by reference). This greatly
restricts the capacity of the external ocular environment to neutralize protease activity by
passive dilution. Moreover, overnight eye closure is associated with the induction of a
subclinical state of inflammation, as evidenced by. . .

et al., Curr. Eye Res. 16:810-819, 1997, each incorporated herein by reference). This results in a stagnant layer enriched in serine proteases and complement products.

The enrichment of serine proteases in closed eye tears raises the question as to how autolytic cell damage is prevented during this period. In further studies,. . . acting as the principal functional entities. These serpins have a combined spectrum of activity such that all known PNIN serine proteases can be inactivated. Sathe et al., Current Eye Res.

the majority of a2-M in C tear fluid from most donors remains intact. Notably, although a2-M reacts with PMN cell proteases, it does so at only a relatively slow rate. Swenson et al., J. Biol.

the period of eye closure, there is a sufficient pool of rapid reacting serpins available so as to quench the released proteases in sufficient time to prevent reaction with a2-M. Sathe et al., Current Eye Res. 348-362, 1998, incorporated herein by reference.

Although the closed eye environment is thus uniquely equipped with protective mechanisms against pathogenic infection, including a battery of fast-acting protease inhibitors, it is nonetheless found that the compositions and methods of the invention are particularly useful in preventing proteolytic. . .

For determining and quantifying the effects of antiproteolytic compositions administered during periods of closed tear production, both animal and human subjects may be used in accordance with the preceding example.. . . same assays as detailed above are employed, with the modification that test and control samples are represented by subjects to whom an antiproteolytic composition is administered, at similar doses and for comparable duration, entirely during a reflex tear or closed eye tear production period, respectively.. . . the associated parameters outlined above. These results thus enable confirmation that the compositions and methods of the invention yield a substantially greater antiproteolytic effect during periods of closed eye tear production compared to periods of reflex tear production.

ii) Antiproteol3qic Activily in Corneal Tissues by Antiprotease Treatment During Periods of Closed Eye and Reflex Tear Production.

In addition to the foregoing analyses, alternate test and control samples are utilized to document that closed eye administration of antiproteolytic compositions of the invention results in quantitative inhibition of proteolysis, not only in the extracorneal fluid, but also in the corneal tissue of treated subjects. For this

purpose, the

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antiproteolytic compositions are administered, as above, to rabbit subjects during closed eye and reflex tear production periods. A protracted treatment regimen is employed, wherein the antiproteolytic composition is administered daily for one week. The subjects are then sacrificed and corneal tissues are isolated and processed for analysis. . .

Protease Extraction From Corneal Tissues

Prior to extraction, corneal tissues are stored in liquid nitrogen. Tissues are then pulverized in liquid nitrogen using. . .

Eparnatic Assgys

Proteases present in soluble protein preparations from corneal tissues of treated and control subjects are analyzed and quantified by assaying against nitrophenyl acetate as a substrate and by substrate (e.g., gelatin) eletrophoresis. Smith et al., Eye 9:429-433, 1995, incorporated herein by reference. All proteases possess acyl transferase activity and will catalyze the liberation of 4-nitrophenol from 4-nitrophenyl acetate.

Results from the foregoing assays enable confirmation that the compositions and methods of the invention yield an antiproteolytic effect in both extracomeal fluids and corneal tissues, which effect is significantly enhanced during periods of closed eye tear production compared to. . .

iii) Prevention of Pathogenic Structural Changes in Keratoconic Corneal Tissues by Antiprotease Treatment During Periods of Closed Eye and Reflex Tear Production.

Additional assays are also utilized herein to document that administration of antiproteolytic compositions of the invention results in quantitative inhibition of structural degradative changes in the corneas of patients suffering from keratoconus. For this. . . for keratotomy or other surgical procedure during which corneal tissues will be incidentally available for harvest. Prior to the scheduled surgical procedure, antiproteolytic compositions of the invention and placebo formulations are administered to test and normal control groups of patients during closed eye and. . . This treatment protocol involves an even more protracted regimen in order to manifest inhibition or reversal of corneal structural changes, wherein the antiproteolytic composition is administered daily for two to six months prior to surgery. At the time of surgery, corneal tissues are harvested. . .

Preparation of EnZyme

Corneal tissue samples from closed eye and reflex eye subgroups of antiprotease treated and non-treated subjects are each divided into three enzyme assay

sample groups, a normal control group, a trypsin activated test. . .

. .

are fixed in IO% formalin, processed, and embedded in paraffin. Five-micrometer-thick sections are cut, deparaffinized, rehydrated and subjected to primary and secondary antibody incubations.

To detect levels of exemplary protease inhibitors, the rehydrated sections are incubated first in IO% normal goat blocking serum for 20 minutes and then with polyclonal rabbit antiliuman a2-macroglobulin antibodies (1: 1 00 dilution; Dako, Santa Barbara, CA) for 1.5 hours. This affinity-purified antibody preparation is monospecific by I 0 immunoelectrophoresis against plasma. Alternatively or additionally, primary antibodies to a 1 -antichymotrypsin and/or a2-antiplasmin (Athens Research Technology, Athens, GA) are employed. After incubation with the primary antibody and rinsing with phosphate-buffered saline, the sections are further incubated with biotinylated goat anti-rabbit IgG (1:500; Vector Laboratories, Burlinghame, CA) for 30 minutes, and soaked in 0.3% H202-methanol for 20 minutes to block the endogenous. . . the reaction products is examined under light microscopy. For negative controls, normal rabbit IgG is used in place of the primary antibody.

Additional correlative immunohistochemical assays are conducted to determine levels of proteases in the corneal samples. For example, sections are incubated first in IO% healthy goat or healthy rabbit blocking serum for 3 0 minutes and then incubated with polyclonal sheep anti-human cathepsin B (ICN, Costs, Mesa, CA), monoclonal anti-cathepsin G (Pel Freeze Biologicals, Rogers, AK), monoclonal antibodies to MMP-1, MMP-2, MMP-3, MMP-9, TIMP-1, TIMP-2, and plasminogen activator inhibitor- I (Oncogene Science, Cambridge, MA), and/or polyclonal antibodies to TPA and urokinase (American Diagnostica, Greenwich, CT) for 1.5 hours. The dilutions of antibodies range from 1: IO to 1:200. All antibodies are affinity purified and monospecific, as judged by immunoelectrophoresis. Corneal sections serving as negative controls receive the same dilutions of nonimmune sheep immunoglobulin G (IgG), rabbit IgG, mouse IgG, or anti-digoxigenin. After the primary antibody incubation, the tissue sections are allowed to react with biotinylated goat anti -rabbit (1:250), rabbit anti-sheep

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(1:250), or goat antimouse IgG (1:500) for 30 minutes. They subsequently are soaked in 0.3% H202-methanol for 20 minutes to block the endogenous peroxidase and. . .

For Western blot analyses, aliquots of the supernatant fractions (10]tl) and target antigens (e.g., a2-M) are electrophoresed on 6% sodium

dodecyl sulfate gels under reducing conditions. The proteins are electroblotted overnight onto a nitrocellulose membrane. . . NH). - After blocking with 5% nonfat dry milk (Carnation, Los Angeles, CA), the membrane is allowed to react with either the antibody probe (e.g., rabbit anti -a2-macroglobulin--1: 1 0,000; Dako) or IgG (e.g., rabbit IgG-- 1: I 0,000; Cappel, Durham, NC), followed by incubation with secondary antibody (e.g., goat anti-rabbit IgG conjugated to horseradish peroxidase--1:10,000; Cappel).

. . . .

Data from triplicate samples are averaged. Non-specific binding is determined on duplicate blots incubated with only normal rabbit IgG and the secondary antibody. These are subtracted from the total binding to determine the specific binding. Resultant values are then compared with the standard curve obtained. . .

In conjunction with the foregoing assays, antiproteolytic activity may be closely defined in terms of quantitative differences in morphological and/or ultrastructural features attributable to proteolytic activity between test and. . .

By applying the foregoing procedures and principles, it will be shown that the antiproteolytic compositions and methods of the invention effectively treat corneal disease in a mammalian patient. In particular, the assays utilized herein enable demonstration that antiproteolytic compositions of the invention provide quantitative inhibition of structural degradative changes in the corneas of patients suffering from keratoconus. Correlative. . . with substantial reduction of specific proteolytic activity within targeted corneal tissues, which effects are in turn attributable to the activity of exogenous protease inhibitors administered according to the methods of the invention. Within these methods, preferred embodiments involve administration of the inhibitor(s) to a subject. . .

EXAMPLE III

FORMULATIONS FOR TOPICAL OCULAR DELIVERY OF PROTEASE INHIBITORS

A variety of topical antiprotease formulations are useful within the methods of the invention, including solutions, gels, creams, particulate suspensions, and the like. For example, eyedrops comprising an aqueous protease inhibitor solution isotonic with tears are particularly useful for repeated instillation during periods of reflex tear production. However, poor bioavailability of drugs. . . attributable to a variety of factors, including rapid clearance of therapeutic agents by tear flow, neutralization or degradation of therapeutic agents by proteases and other mechanisms,

barriers to diffusion and penetration of therapeutic agents across the corneal epithelium to
50
reach the corneal stroma and other. . .

i) Mucoadhesive Polymer Formulations
To address the foregoing delivery issues, the invention provides protease
inhibitor compositions wherein the protease inhibitor is dissolved, suspended, or admixed
with a delivery vehicle or carrier comprising an opthalmically acceptable, mucoadhesive
polymer. Mucoadhesive polymers that adhere. . . each incorporated herein by
reference. As used within the compositions and methods of the invention, mucoadhesive
carriers prolong contact between the subject protease inhibitor(s) and the underlying
absorptive tissue. At the same time, the mucoadhesive carrier ftinctions integrates with,
or replaces, naturally adhering mucin until the latter is replenished, which intensified
contact weakens barrier properties of the epithelium and results in facilitated penetration
of the protease inhibitor(s). These properties of mucoadhesive polymer delivery systems
within the invention can be readily determined and optimized in accordance with the
following. . . protocols all specifically demonstrate that
mucoadhesive polymers, e.g., polycarbophil (a derivative of polyacrylic acid loosely
cross-linked with divinylglycol) improves ocular delivery of protease inhibitors in an
accepted model system for corneal delivery using the pigmented rabbit.
.
be obtained
from a variety of commercial sources. For the present example, Polycarbophil (Noveon
AA-1) is obtained from BF Goodrich (Cleveland, OH). Protease inhibitors are obtained
from various sources, as exemplified above. Protease inhibitor levels are measured by
any of the foregoing methods or, alternatively by fluorescence polarization immunoassay
using a TDx system (Abbott Laboratories,. . .

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Preparation of Test Formulations
Multiple test formulations are prepared by mixing a selected protease
inhibitor with various vehicles, including (1) a 0. 8 1 % (wt/vol) NaCl solution; (2) 0.8 1 %
(wt/vol) NaCl and 4.5%. . .

Any one or more of the above enumerated protease inhibitors may be
included in the mucoadhesive formulation. In the present example, a2-M and alp I are
combined in a series of. . .

Release of Protease Inhibitor In Vitro
Fifty-microliter aliquots from each formulation are added to 5 ml of
glutathione-bicarbonate Ringer's solution of pH 7.4 in a. . . for 2 minutes to sediment the polymer
particles. A 50]tl sample is withdrawn from the supernatant and assayed

to determine concentrations of protease inhibitor(s) after making a 20-fold dilution with TDx-buffer.

. . .

to affect the extent of ocular absorption of topically applied pilocarpine, epinephrine, and

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chloramphenicol. Even if this were not the case for protease inhibitors, comparative evaluations of different delivery formulations should not affected.

Rabbits are kept upright in minimally restraining cases throughout the experiment. The protease inhibitor formulations (25]tl) are instilled using an Eppendorf pipette (Brinkman Instruments, New York, NY) at the upper lid of each. . .

To determine the role of the corneal epithelium in corneal penetration of protease inhibitors, the corneal epithelium is removed before administration of the mucoadhesive formulation by carefully scraping with a lancet blade under topical anesthesia. . .

Aqueous humor and vitreous humor samples are assayed directly utilizing appropriate immunological assays, whereas corneal tissues are subjected to initial processing and protease extraction, as described above.

In accordance with the above methods, the invention provides novel delivery systems comprising a protease inhibitor dissolved or suspended in a mucoadhesive polymer. These compositions are readily optimized in terms of consistency and inhibitor concentration so as to maximally prolong ocular residence time and enhance transport of protease inhibitors and coordinately administered agents to intraocular target sites (e.g., corneal epithelia, stroma, Bowman's layer and vitreous humor compartments). In addition, the. . .

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ii) Microparticulate Delively Formulations

Although mucoadhesive polymer formulations provide useful vehicles for prolonged ocular delivery of protease inhibitors, microparticulate technology is also shown to provide preferred, long-term ophthalmic delivery vehicles for use within the invention. Methods of the invention which utilize microparticulate formulations as delivery vehicles for antiproteases are particularly amenable to use during periods of closed eye tear production (e.g., overnight use), further optimizing these novel methods of the. . .

. . .

are small polymeric particles (erodible, non-erodible or ion exchange resins) suspended in a liquid carrier medium. These particles contain one or more protease inhibitors in an antiproteolytic effective concentration. Upon administration of the microparticulate suspension in

the eye, the particles reside at the delivery site (cul-de-sac, sub conjunctiva or vitreous cavity) and the protease inhibitor(s) is released from the particles through diffusion, chemical reaction, polymer degradation, or ion-exchange mechanism.

. . .

can also be employed for treating corneal disease according to the methods of the invention, whereby release of effective levels of protease inhibitors can be sustained for long-term periods of 2-3 days and up to two weeks or more. Both corneal and non-corneal. . .

Two general microparticulate forms employed for ophthalmic delivery of antiproteases are microcapsules and microspheres. Particles or aqueous droplets of proteases are entrapped inside a polymeric membrane of microcapsules, yielding almost spherical entities on the order of several hundred microns in diameter. Microspheres are polymeric combinations where the protease inhibitor is homogeneously dispersed in the polymer matrix. Nanoparticles possess similar characteristics of microspheres except that their size is approximately three orders. . .

. . .

eye dropper, an attractive feature for patient acceptability and compliance. For intraocular application, the microparticulate suspension has an advantage of deliverability of antiproteases to the target site through a 27 gauge needle, rather than an involved surgical procedure that is required for device implants.

Time Constant for Chemical Reaction

Release of protease inhibitors through bioerodible polymers is a function of the rate of hydrolysis of the acid or base labile linkages of the polymer and the subsequent release of the protease inhibitor via diffusion through the pores of the matrix created by the polymer hydrolysis. As expected, the time constant for chemical. . .

Microparticulate systems rely on release of protease inhibitors and other therapeutic agents due to diffusion, reaction, physical erosion and ion exchange at the delivery. Each physical or chemical event. . .

. . .

may stimulate reflex tear rate varying between 3 RI to 400 RI per minute. Blinking of the eyelids facilitates spreading of the protease inhibitor in the lacrimal pool over the cornea.

. . .

release of the inhibitor that is predicted or extrapolated by in vitro release kinetics experiments. The primary route by which the protease inhibitor reaches the site of action is the cornea (Lee et al., J. Ocular Pharmacol. 2:67-108, 1986, incorporated herein by reference). However,. . .

The following different approaches can be used to formulate protease inhibitors (and other drugs in combinatorial formulations) in microparticulate dosage form for topical administration.

Polymer-Inhibitor CoMplex

Polymer-inhibitor complexation involves covalent or chemical linking of the protease inhibitor(s) to the polymer backbone forming a macromolecule pro drug.

. . .

ophthalmic delivery has been described. Lee et al., J. Ocular Pharmacol. 6:157-169, 1990, incorporated herein by reference. In the present example, the antiprotease is homogeneously dispersed (monolithic system) in the polymer matrix. As such, the loaded microparticles are then suspended in a liquid carrier medium, which may also include the protease inhibitor.

These polymers are suitable for release of proteins of different sizes, including full length protease inhibitors and active polypeptide fragments of protease inhibitors.

. . .

acid dimer and sebacic acid of varying molecular weights may be copolymerized for microsphere preparation. The in vitro release of I 0 protease inhibitors from microspheres depends upon the particle size, cross-linking density and protease inhibitor loading. Within the formulations of the invention, near constant or zero order release rates for one week or more can. . . .

. . .

acid (L and D form) and liquid crystalline polymer (polyoxyethyelene glycerol tristearate) in microsphere dosage form may also enhance ocular delivery of protease inhibitors. These compositions have inherent ability to modulate inhibitor delivery/release rates in response to small temperature changes in the envirom-nent. Thermoresponsive microspheres. . . .

In-Situ Gelliny, Latex Nanovarticles

These systems are comprised of antiprotease impregnated nanoparticles suspended in a carrier medium and ready to be directly administered into the eye. The protease inhibitor can be either chemically bound to the polymer backbone or entrapped in the polymer matrix. The preparation of latex particles. . . .

Ion-exchanize

Long-term delivery rates are difficult to achieve for delivery systems for which diffusion limits the release of the antiprotease. The time constant for diffusion is dictated by diffusional length of the diffusing species, which is small for ophthalmic systems because of. . . .

The diffusivity of antiproteases in the polymer can be changed, but to have a significant impact on delivery times and rates requires change of diffusivity. . .

Ion exchange delivery systems provide a suitable alternative to circumvent the limitations of diffusional dependent antiprotease release. The availability of free protease inhibitor for diffusion is limited by the exchange kinetics between the inhibitor and the ion exchange resin and the adsorption isotherm.. . .

. . .

is\circumvented by fabricating microparticles with molten method technique. However, this technique is plagued by the stability issue relating to both the protease inhibitor and the polymer. The inhibitor is incorporated in the polymer by preparing polymer melt at as high as 180'C. Exposure. . .

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the foregoing procedures and principles, it can be readily demonstrated that a macromolecular pro drug delivery system provides for prolonged delivery of protease inhibitors, while achieving lower peak inhibitor concentration and consequently resulting in reduced systemic side effects and higher bioavailability. These delivery systems are useful for sustained ocular delivery, of protease inhibitors (e.g., 4-8 hr, preferably 8-12 hour, more preferably 12-24 hr) and are also adaptable for long term delivery (e.g., multiple days and up to one to two weeks). Notably, these delivery vehicles can be readily adapted for optimal delivery of protease inhibitors to the eye to treat corneal disease during periods of closed eye tear production.

EXAMPLE IV

USE OF PRESERVATIVES WITH TOPICAL ANTIPROTEASE FORMULATIONS: PERMEABILITY AND TOXICITY

Deep corneal and intraocular delivery of protease inhibitors is complicated somewhat by the large size of these proteins. Whereas active peptide fragments of protease inhibitors may be efficiently delivered to intraocular compartments, it is more

difficult to achieve the same level of penetration with full length antiprotease agents.

This problem can be addressed by various modifications of antiproteases, for example by converting the inhibitor to a derivative or salt having higher permeability characteristics, or admixing or conjugating the inhibitor to. . .

. . .

be particularly useful within the invention include multifaiictional penneabilizing agents. As used herein, multifunctional perineabilizing agents enhanced corneal and intraocular delivery of protease inhibitors and also provide a second, preservative or antibacterial ftmction to the antiprotease 1 5 composition. In this regard, the present example provides methods for selecting

preferred, multifunctional permeabilizing agents for use within antiprotease. compositions.

In order to evaluate the usefulness of antiprotease formulations containing ophthalmic preservatives, concentrations of the protease inhibitor in tears and corneal tissues are determined for formulations with and without a candidate preservative, before and after instillation of the. . .

Materials Protease inhibitors, e.g., a2-M and alpl, are provided as set forth above. Saponin is obtained from E. Merck (Darmstadt, Germany). Sorbic acid (SA), ...

In Vivo Instillation Studies
Unanesthetized rabbits are constrained in a prone position, and twenty-five]d of an antiprotease formulation (ranging in concentration as above) containing preservatives (0.01% BK, 0.04% PR, 0.5% PE, 0.5% BA and 0.25% SA) is carefully applied. . .

Concentrations of protease inhibitors in the tears and corneal tissues is determined by enzyme and/or immunological assays, as above.

Local Toxicity and Irritation
For determining local toxicological effects, rabbit eyes are gently washed 6 h after instillation of the antiprotease formulation. The eyes are subsequently examined and scored according to the scale of Draize et al. J. Pharmacol. En. The .. . .

Blinking counts are measured as a local irritation index after instillation of the antiprotease formulation containing preservatives (0.01% BK, 0.04% PR, 0.5% PE, 0.5%BAandO.25%SA). ApH7.4bufferisusedasacontrol. 0.1%saponinandO.1% EDTA are also used as an absorption promoter control. Blinking. . ..

The foregoing protocols allow demonstration that antiprotease fort-nulations containing ophthalmic preservatives delivered via an ocular route increase the penneability of ocularly administered antiproteases. Thus, these formulations are particularly useful within the invention by their ability to simultaneously stabilize fon-nulations, inhibit microbial infection, and enhance permeability and efficacy of antiproteolytic agents.

CLMEN. . . method for treating corneal disease in a mammalian subject comprising administering to an ocular fluid, surface, or tissue of the subject an antiproteolytic effective amount of a protease inhibitor in an opthalmically acceptable carrier.

2 The method of claim 1, wherein the protease inhibitor is selected

from an aspartic, serine, cysteine, or metallo-protease inhibitor.

3 The method of claim 1, wherein the protease inhibitor is recombinantly or synthetically modified.

4 The method of claim 1, wherein the protease inhibitor is administered topically.

6 The method of claim 1, wherein the protease inhibitor is administered during a period of closed eye tear production by the subject.

1 5 7. A device for corneal delivery of an effective amount of an antiprotease comprising:
an ocular implant formed of an opthalmically acceptable material and having a concave inner surface sized and dimensioned to conform to an external surface of a human cornea, said inner surface coated or impregnated with an protease inhibitor.

sized and dimensioned to conform to an external surface of a cornea of the subject and coated or impregnated with an protease inhibitor in contact with the cornea for an effective treatment period sufficient to achieve delivery of said protease inhibitor to an ocular fluid, surface, or tissue of the subject.

11 A pharmaceutical formulation for treating corneal disease in a mammalian subject comprising an antiproteolytic effective amount of a protease inhibitor in an opthalmically acceptable carrier.

12 The pharmaceutical formulation of claim 1 1, wherein the protease inhibitor is selected from an aspartic, serine, cysteine, or metallo-protease inhibitor.

13 The pharmaceutical formulation of claim 1 1, wherein the protease inhibitor is recombinantly or synthetically modified.

14 The pharmaceutical formulation of claim 1 1, wherein the protease 5 is formulated in a carrier adapted for topical administration selected from a topical solution, cream, gel, or microparticulate carrier.

16 The pharmaceutical formulation of claim I 1, comprising a plurality of protease inhibitors.

17 The pharmaceutical formulation of claim 16, comprising a plurality of multispecific protease inhibitors.

18 The pharmaceutical formulation of claim 17, wherein at least one of said protease inhibitors is selected from a2-M, alp 1, or SLP 1.

19 The pharmaceutical formulation of claim 16, comprising a first, multispecific protease inhibitor and a second, oligospecific or specific protease inhibitor.

. The pharmaceutical formulation of claim 19, wherein at least one of said protease inhibitors is selected from a2-M, alpl, SLPI,

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PI-antigellagenase, a2-
  antiplasmin, serine amylyoid A protein, al-
antichymotrypsin (al-Achy), cystatin C,
inter-a-trypsin inhibitor, elafin, elastinal, aprotinin, phenylmethyl
sulfonyl fluoride,
leupeptin, TIMP- 1, TIMP-2, or 1, I 0-phenanthroline.
. . .
claim I 1, further comprising a
second therapeutic agent selected from a permeabilizing agent, a
preservative agent, a
stabilizing agent, or an antibiotic, antiviral, or
antiinflammatory drug,
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ACCESSION NUMBER:         2001030380 PCTFULL  ED 20020820
TITLE (ENGLISH):          METHODS AND COMPOSITIONS FOR TREATMENT OF KERATOCONUS
                          USING PROTEASE INHIBITORS
TITLE (FRENCH):           TECHNIQUES ET COMPOSITIONS DESTINEES AU TRAITEMENT DU
                          KERATOCONE AU MOYEN D'INHIBITEURS DE PROTEASE
INVENTOR(S):              QUAY, Steven, C.
PATENT ASSIGNEE(S):       K-QUAY ENTERPRISES, LLC;
                          QUAY, Steven, C.
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PATENT INFORMATION:

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                            WO 2001030380        A2 20010503
DESIGNATED STATES
       W:                 AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU
                          CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN
                          IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK
                          MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM
                          TR TT TZ UA UG US UZ VN YU ZA ZW GH GM KE LS MW MZ SD
                          SL SZ TZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY
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APPLICATION INFO.:        WO 2000-US29229       A  20001020
PRIORITY INFO.:           US 1999-60/161,879       19991027
TIEN   METHODS AND COMPOSITIONS FOR TREATMENT OF KERATOCONUS USING
       PROTEASE INHIBITORS
TIFR   TECHNIQUES ET COMPOSITIONS DESTINEES AU TRAITEMENT DU KERATOCONE AU
       MOYEN D'INHIBITEURS DE PROTEASE
PI     WO 2001030380        A2 20010503
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ABEN Compositions and methods for treating corneal diseases mediated by elevated protease activity include ocular administration of protease inhibitors. One or more protease inhibitors selected from an aspartic, serine, cysteine, or metallo-protease inhibitor are administered to an ocular fluid, surface, or tissue, preferably by topical administration, to inhibit proteolytic activity associated with. . . for example keratoconus. Antiproteolytic formulations of the invention may include carriers that prolong the retention and/or enhance delivery of the protease inhibitor. These formulations can also include other therapeutic agents such as antiinflammatory or antibiotic drugs. In preferred aspects of the. . . during periods of closed eye tear production. Also provided within the invention are implant devices for corneal delivery of a protease inhibitor.

DETD METHODS AND COMPOSITIONS FOR TREATMENT OF KERATOCONUS USING PROTEASE INHIBITORS

BACKGROUND OF THE INVENTION

Keratoconus is a bilateral ocular disorder that progressively thins and distorts the central portion of the cornea toward. . .

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regard, it has long been noted that keratoconic corneas exhibit various ultrastructural defects, including fragmentation of Bowman's layer, fragmentation of the epithelial cell basement membrane, and fibrillation of the anterior stroma. Teng, Am. J. Oplithalmol. 55:18-47, 1963; Chi et al., Am. J. Oplithalmol. 42:847-60, 1956;. . .

. . .

thickness of Bowman's layer remains unchanged. Components of Bowman's layer are believed to be synthesized by both corneal epithelial and strornal cells, and an epithelial-stromal interaction is suggested to be a major factor in the formation of Bowman's layer. Hay, Int. Rev. Cytol. 63:263-322,. . . .

. . .

fibrils in Bowman's layer are of relatively small diameter and are randomly

3

arranged. In the underlying comeal stroma, the resident strornal cells are responsible for the maintenance and organization of the collagens. However, considering that Bowman's layer is acellular, the organization and maintenance of collagens at this site remains unexplained. One possibility is that these ftinctions are performed by the sparse strornal cells that transverse into Bowman's layer. Cytokines have also been suggested to play a role in collagen maintenance in Bowman's layer. In keratoconus, the collagen maintenance function of stromal cells and/or cytokines may be disturbed for both Bowman's layer and for the stroma.

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reports conclude that keratoconic tissues exhibit higher levels of protein and increased incorporation of protein precursors (e.g., [3 H]-proline) into all cell layers compared with normal comeal tissues. Rehany et al., Invest. Oplithalmol. Vis. Sci.

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model, Yue et al., Proc. Soc. Exp. Biol. Med. 175:336-341, 1984, incorporated herein by reference, report that corneal

specimens and cultured
cells from affected persons show a reduction in overall protein levels compared with normal controls. However, collagen content is normal in some. . .

107:929-936, 1982, incorporated herein by reference. In light of these reports, some investigators propose that imbalances in protease/ protease inhibitor levels may contribute to decreased protein levels and increased proteolytic activities associated with keratoconus. Additional investigations have reported decreases in the levels of certain proteolytic enzyme inhibitors, for example, a I -protease inhibitor (alp 1) (Whitelock et al., Invest. Oplithalmol. Vis. Sci. 38:529-534, 1997; Sawaguchi et al., En. Eye Res. 50:549-5545 1990, each. . .

However, the model of a protease/protease inhibitor imbalance as a pathogenic mechanism in keratoconus remains speculative. In this regard it is noteworthy that Yue et al., supra, conclude. . . cases where collagen levels are actually reduced, the reduction is apparently due to decreased collagen synthesis rather than to an increase in protease activity or a decrease in protease inhibition. Other reports suggest that pathogenic changes associated with keratoconus, including increased collagenolytic activity, may be attributed to structural changes in the collagen proteins themselves, as opposed to altered collagen expression or elevated protease ftmction (e.g., attributable to higher protease levels or activities, or decreased protease inhibitor levels or activities).

Data obtained by assaying acyl transferase activity show that MMPs account for at least 95% of the total protease secreted by cultured keratocytes. The summated specific activity of MMI's is reported to be consistently and significantly higher in the culture media. . .

Biophys. Res. Commun. 107:929-936,1982; Rehany et al., Ann. Opthalmol.: 14:751-754, 1982, each incorporated herein by reference) and cell culture (Ihlainen et al., Eur. J. Clin.
. .
and casein are preferred substrates for gelatinases A and B, and stromelysin. They can, however, also serve as substrates for other proteases.

To determine whether gelatinolytic and caseinolytic activities associated with keratoconus are caused by MMPs or other classes of proteases, Zhou and coworkers (1998, supra) also employed inhibitors specific for four classes of proteases (aspartic, serine, cysteine, and metallo-proteases) as blocking reagents. These studies reportedly showed that, in both healthy controls and keratoconic specimens, the net gelatinolytic and caseinolytic activities were related mostly to serine and cysteine

proteases, and not to
aspartic proteases, gelatinases A and B, or stromelysin. The inhibitor of serine proteases phenylmethyl sulfonyl fluoride, the cysteine protease inhibitor E-64, and the cathepsin 13-trypsin inhibitor leupeptin substantially reduced digestion of gelatin and casein, whereas the aspartic protease inhibitor pepstatin and the MMP inhibitor 1, I 0-phenanthroline failed to block the reaction.

7
These in situ zymographic results may be contrasted with earlier organ culture and cell culture studies which suggested that increased gelatinase activities in keratoconus are caused by gelatinases A and B and stromelysin. Kat et.

. . .

Zhou and coworkers suggest that most gelatinolytic and caseinolytic activities in healthy human comeas may not be caused by MMPs. Instead, cysteine proteases, such as cathepsin B, and serine proteases, such as cathepsin G, both of which are reportedly elevated in keratoconus, are proposed to contribute to the enhanced gelatin- and casein-digesting. . .

Chem. 267:21830-21838, 1992, incorporated herein by reference). Evidence presented in these and other reports suggests that certain protease inhibitors are reduced in association with keratoconic disease. For example, a decrease in al-protease inhibitor levels were reported in both the epithelial and the stromal layers of keratoconus comeas based on immunostaining and dot blot assays.. . . the stromal layer was confined to the stromal larnellae, and no reduction in the inhibitor level actually occurred in the keratoconus strornal cells. Other evidence based on measurements of rnRNA levels suggest that the a- I -protease inhibitor may be downregulated in association with keratoconus. Whitelock et al. (Invest. Opthalmol. Vis. Sci. 38:529-534, 1997, incorporated herein by reference).

. . .

Despite the uncertainties apparent from these reports, a number of patent disclosures purport to teach methods for treating corneal disease using protease inhibitors.

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Levy et al., and U.S. Patent No. 5,892,112, issued April 6, 1999 to Levy et al., state that synthetic, small molecule, non-peptide protease inhibitors may be useful in treating keratoconus. However, these disclosures add nothing to the foregoing reports with respect to identifying the. . . potential treatment agents and modalities for clinical use against the disease. Moreover, each of these disclosures proposes that vast numbers of synthetic protease inhibitors may be used to treat a laundry list of diseases.

Thus, both of the Levin et al. patents identify hundreds of synthetic protease inhibitors

and generally assert that the disclosed compounds can be used to treat such diverse diseases and conditions as arthritis, tumor growth. . .

Most notably, none of the foregoing patents directed toward production and use of small molecule protease inhibitors for disease treatment provide specific direction or guidance as to the underlying mechanisms of keratoconus and other corneal diseases, nor. . .

Further discussion regarding the possible roles of proteases and their inhibitors in keratocomis is also provided by Kenney et al., Biochem. Biophys. Res.

. . .

Chem. 264:1860-1869, 1989,

9

incorporated herein by reference. Also, unlike collagenase and stromelysin, levels of gelatinase expression are not enhanced by exposure of cells to tumor promoter 12 tetradecanolyphorbol 13-acetate, but are increased by transformation with H-ras oncogene and treatment with transforming growth factor-P.

Based on these results, Kenney et al. propose an alternative mechanism to the protease/protease inhibitor imbalance model proposed by others. In particular, the authors suggest that there may be an inherent difference between the diseased. . .

Reported changes in protease and protease inhibitor properties (e.g., expression, structure, activity or metabolism) are difficult to interpret, and to reconcile with other reports.

. . .

of keratoconus, or merely represent incidental sequelae of the disease. Resolution of these alternative possibilities is further complicated by the possibility that protease and protease inhibitor activities may be altered in keratoconus as a result of concurrent wound-healing and repair mechanisms, allergic responses, or responses to mechanical trauma, rather than as a direct effect of the primary disease. For example, declines in the protease inhibitor levels in the cornea may simply be a reflection of a similar decline in tears, or in serum.

. . .

Endocrinology 108:2129-2135, 1981) and allergic disease (Berman et al., Invest. Opthalmol. Vis. Sci. 12:759-770, 1973, each incorporated herein by reference) can affect protease inhibitor levels in tears and in serum, and allergic diseases are often associated with keratoconus (Rahi et al., J.

. . .

may be influenced by a variety of complex molecular and biochemical factors. In this context, recent discussion has been directed toward specific proteases as potential modulators of

proteoglycan structure/metabolism. Cathepsin B, a cysteine protease, and cathepsin G, a neutral serine protease, are both enzymes known to degrade proteoglycans in the corneal stroma, which is the site of thinning and scarring in keratoconus.. .

. . .

62:325-337, 1996, incorporated herein by reference, have postulated that interleukin I (IL-1) may be a cytokine modulator of epithelial-stromal interactions, regulating corneal cell proliferation, differentiation, and cell death. These investigators have also proposed a causal role of the IL-1 system in keratoconus. Support for this model includes findings that cultured keratoconus stromal cells contain 4-fold higher binding sites for IL Fabre et al., Curr.

. . .

interface between the corneal epithelium and stroma. Components of Bowman's layer are thought to be synthesized by both corneal epithelial and strornal cells, and maintenance of this layer is believed to require complex epithelial-stromal interactions.

. . .

the role of collagen maintenance in the cornea. In this context, it has been proposed that the collagen maintenance function of stromal cells and/or cytokines may be disturbed for both Bowman's layer and for the stroma. However, the organization and maintenance of collagens in. . .

The methods of the invention involve ocular administration of an antiproteolytic effective amount of a protease inhibitor in an opthalmically acceptable carrier. The protease inhibitor is preferably a protein or peptide protease inhibitor selected from an aspartic, serine, cysteine, or metallo-protease inhibitor, which may be derived from a natural source or produced in a native or modified form by recombinant or synthetic techniques known in the art. In most instances, recombinant or synthetic protease inhibitors are preferred, as these materials will generally be free of undesirable contaminants and infectious agents.

According to the methods of the invention, a protease inhibitor formulation is administered to an ocular fluid, surface, or tissue, preferably by topical administration, in an antiproteolytic effective amount to substantially. . . with the corneal disease or condition to be treated. Proteolytic activities in this context may include activities of multispecific or specific proteases, complex formation between a protease and protease inhibitor, histopathological changes in the cornea attributed to proteolytic processes, and other indicia correlated with proteolytic mechanisms.

. . .

a single agent that enhances permeability and provides a simultaneous preservative function. In addition, the formulation can include a plurality of protease

inhibitors, as well as other therapeutic agents such as antfinflaminatory or antibiotic drugs.

. . .

a corneal disease or condition and serves as a carrier to deliver to the cornea an antiproteolytically effective amount of a protease inhibitor.

. . .

disease in a mammalian patient. The methods of the invention involve ocular administration of an antiproteolytic effective amount of a

I 0 protease inhibitor in an opthalmically acceptable carrier. The protease inhibitor is preferably selected from an aspartic, serine, cysteine, or metallo-protease inhibitor, obtained from a natural source or produced in a native (i.e., wild-type amino acid sequence) or modified (e.g., by amino acid. . .

According to the methods of the invention, a protease inhibitor formulation is administered to an ocular fluid, surface, or tissue, preferably by topical administration, in an antiproteolytic amount effective to substantially. . .

By substantial inhibition of proteolytic activity is meant that administration of the protease inhibitor formulation yields at least about a I0% reduction of proteolytic activity, preferably at least a 20% reduction, compared to a. . . target site, for example within the extracomeal fluid, corneal epithelium, corneal stroma, Bowman's layer, or the vitreous humor. Preferably, administration of the protease inhibitor yields approximately a 30-50% reduction of proteolytic activity, more preferably greater than about a 50% reduction, and in some preferred aspects. . .

As used herein, proteolytic activity refers to a quantitative digestive activity of a target protease against a protein (e.g., collagen, elastin, fibronectin) or glycoprotein (e.g., a proteoglycan or glycosaminoglycan) substrate. Target proteases as herein defined include proteolytic enzymes that exhibit aberrantly high levels of expression or activity (e.g., attributable to structural changes that increase substrate

1 8

binding or otherwise enhance digestion kinetics, or that render the protease more susceptible to activation from a proenzyme to an active form), or whose regulation (e.g., by metabolic turnover, protease inhibition or other mechanisms) is impaired in association with a corneal disease or condition to be treated, for example keratoconus or corneal infections. As further defined herein, target proteases are amenable to regulatory inhibition by exogenously administered protease inhibitors.

Proteases that may be successfully targeted for inhibition by the compositions and methods of the invention include, but are not limited to,. . .

. . .

the invention. Exemplary indicia in this context include quantitative changes in the extent of fragmentation of Bowman's layer, fragmentation of the epithelial cell basement membrane, and/or fibrillation of the anterior corneal stronia. These indicia can be readily compared between treated samples and relevant control samples,. . .

. . .

and control samples include cultured, normal and keratoconic keratocytes, respectively, each treated with an antiprotease formulation of the invention. Using these samples, protease inhibition can be measured at selected time points, for example, by assaying target protease-inhibitor complex formation, rates or levels of protein digestion attributed to the target protease,

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morphological indicia as noted above, and other parameters consistent with the quantitative values sought.

. . .

with keratoconic and normal corneas to provide, respectively, test and control samples for in vitro assays. For quantitative determination of in vivo protease inhibition, test and control samples may include extracorneal fluid or corneal tissue samples taken from subjects (e.g., a human or non-human mammal such as a rabbit) following administration of a protease inhibitor formulation (test sample), and following administration of a placebo comprising, e.g., a selected carrier without the protease inhibitor (control sample). Often, test and control samples will be provided by bilateral administration of test and control treatments to an individual. . .

Protease inhibitors that are useful within the invention are any of the inhibitors, their analogs, recombinantly modified variants, proteolytically active fragments, derivatives, or salts, which can inhibit tarizet proteases as defined above.

. . .

herein that provide for enhanced absorption, retention and delivery of the inhibitor at a site of treatment. In various preferred embodiments, the protease inhibitor may be selected from an aspartic, serine, cysteine, or metallo-protease inhibitor. Useful inhibitors may be derived from a natural source or produced in a native or modified fonn by recombinant or synthetic techniques known in the art. In more detailed aspects of the invention, protease inhibitors bind with one or more proteases that exhibit increased levels of expression or activity, or aberrant regulation, leading to pathogenic protein or glycoprotein degradation and/or morphologic changes associated with. . .

As noted above, preferred protease inhibitors include native or modified aspartic, serine, cysteine, or metallo-protease inhibitors. Exemplary inhibitors in this context include al-antiprotease (alpl, formerly known as al-antitrypsin), a2-macroglobulin (a2-M), secretory leucocyte protease inhibitor (SLP I, formerly known as mucus proteinase inhibitor and antileukoprotease), 0 1 -antigellagenase, a2-antiplasmin, serine amylyoid A protein, al -antichymotrypsin (al -Achy), cystatin C, inter-a-trypsin inhibitor, elafin, elastinal, aprotinin, phenylmethyl sulfonyl fluoride, the cysteine protease inhibitor E-64, the eathepsin B-trypsin inhibitor leupeptin, and the metalloprotease inhibitors TIMP-1, TIMP-2, and 1, 10-phenanthroline.

A particularly preferred protease inhibitor for use within the compositions and methods of the invention is a2-maeroglobulin. This inhibitor is a high-molecular-weight (718 W), homotetrameric glycoprotein implicated as a regulator of degradation for certain extraeellular matrix components and other macromolecules. Unlike many other protease inhibitors, a2-maeroglobulin is not highly specific for a preferred target protease, and is not particularly fast acting. Instead, a2-macroglobulin inhibits proteases from all four major classes and is considered to be relatively slow in its activity.

Consistent with these properties, the mechanism of action by a2-macroglobulin is also unique. When this protease inhibitor reacts with a target protease, proteolytie cleavage in the bait region of the inhibitor occurs, leading to a eonformational change and trapping of the protease. A covalent bond is then formed between the protease and a2-macroglobulin. The protease-inhibitor complex is ultimately cleared from the circulation by a receptor-mediated mechanism.

Another preferred protease inhibitor for use within the compositions and methods of the invention is a-1 protease inhibitor (alpl), a major protease inhibitor in human plasma synthesized mainly by parenchymal liver cells. Alp I is a glycoprotein of 53 kDa that forms a 1: I complex with its target enzyme, leukocyte elastase. In. . . inhibits chymotrypsin, cathepsin G, trypsin, plasmin, and thrombin. It is present in most body fluids, as well as in many tissues and cells. Alp I has been demonstrated in all three layers of normal cornea as well as in the tears and aqueous humor.

Pharmaceutically acceptable derivatives and complexes of protease inhibitors include native or modified inhibitors that are chemically modified (e.g., by addition of stabilizing or otherwise functional chemical moieties),

truncated, conjugated (e.g.,. . .

Particularly useful in this context are protease inhibitor analogs, which comprise recombinantly modified variants and proteolytically active fragments of native inhibitors. These analogs preferably exhibit at least 80% amino. . .

For practicing the methods of the invention, the precise amounts of protease inhibitors to be administered and the frequency and duration of treatment will depend on the status of the corneal condition or.. . .

In a preferred aspect of the invention, compositions comprising a protease inhibitor are administered during periods of closed eye tear production (e.g., during a patient's sleep periods). This method greatly enhances. . . antiproteolytic results, yielding prolonged inhibition of proteolytic processes in corneal tissues (e.g., as demonstrated by reduction in the activity of specific target protease(s)), and long-term inhibition of histopathological changes, such as fragmentation of Bowman's layer. Administration of the antiprotease compositions of the invention during periods. . . attributable to fundamental differences in the processes and regulation of proteolysis that characterize the closed eye, versus reflex tear environments. The protease inhibitor compositions and pharmaceutical formulations of the invention can also be administered during a period that is concurrent with or closely preceding. . .

Within the methods of the invention, formulations comprising a protease inhibitor, a mixture of a plurality of protease inhibitors, or a mixture of one or more protease inhibitors combined with a second therapeutic agent (e.g., an antibiotic, antiviral or antiinflammatory drug) can be administered by a variety of. . . . . . .

contemporaneous to yield coordinate treatment) administration of a plurality of antiprotease proteins, analogs, salts, or derivatives, or administration of formulations comprising multiple protease inhibitors that may be admixed or complexed. Practice of these methods reduces abnormal proteolytic mechanisms attending a targeted corneal disorder (e.g., keratoconus) at combinatorial antiproteolytic levels that exceed antiproteolytic levels observed when either of the coordinately administered protease inhibitors are administered alone. This inhibition, as when other compositions and methods of the invention are employed, reduces proteolytic activity in extracomeal fluid (tears), and in corneal tissues (as determined by both enzymatic and histopathological assays). In preferred embodiments, a multispecific protease inhibitor (i.e., an inhibitor which targets multiple protease species), such as a2-M, SLP I

and alp 1, is coordinately
administered with another multispecific inhibitor, or, alternatively, a multispecific
inhibitor is. . . inhibitors TIMP- 1, TIMP-2, and 1, I
0-phenanthroline). Using these
combinatorial compositions and treatment methods, the invention achieves effective
inhibition against multiple proteases (and/or their pathogenic effects) involved in a
particular corneal disease process. Thus, the methods and compositions of the invention
provide antiproteolytic effects against a broad range of proteases, including but not
limited to, acid esterases, acid phosphatases, acid lipases, cathepsins, collagenases,
elastases, tryptases, chymases, kinins, kalikreins, tumor necrosis factors, chymotrypsins,
26
stromelysins,. . .

Additional preferred methods of the invention involve coordinate administration of an antiprotease and an antibiotic, or administration of fon-nulations
comprising both a protease inhibitor and an antibiotic. Practice of these methods reduces
abnormal proteolytic mechanisms attending a targeted corneal disorder (e.g., keratoconus)
and also secondarily. . .

Typically, the protease inhibitors, analogs, salts, derivatives, and
coordinately administered therapeutic agents of the invention will be administered in the
form of a pharmaceutical composition,. . .

In other embodiments of the invention, the protease inhibitors, analogs,
salts, derivatives, and coordinately administered therapeutic agents of the invention are
prepared with carriers that protect the compound against rapid. . .

a
corneal disease or condition. The device serves as a carrier to deliver to the cornea an
antiproteolytically effective amount of a protease inhibitor. Preferably, the device is
comprised of a gas-permeable, biocompatible polymer, such as ethylene vinyl acetate,
polyanhydride, polyglycolic acid, collagen, polyorthoester, or polylactic acid. The entire
body, or at least an inner surface, of the device is coated or impregnated with the protease
inhibitor. The device is disposable and provided in sterile packaging, to be implanted by
the patient and worn for a selected treatment. . .

is then evaporated,
leaving behind a thin film of dried lipid on the surface of the container. An aqueous
solution of the protease inhibitor is then introduced into the container. The container is
swirled by hand to free lipid material from the sides of. . .

In preferred methods within the invention, mammalian subjects, including
human patients, suffering from protease-mediated corneal

disorders are treated by administering to the patient a pharmaceutical or therapeutic composition comprising an effective amount of one or more. . .

.

wherein EC50 is the concentration of compound that provides 50%

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inhibition of a target proteolytic activity (e.g., proteolysis by a specific protease, or histopathologic change attributed to proteolysis) compared to a relevant control, and IC50 is the concentration of compound that is toxic to 50% of target cells (e.g., keratocytes in an in vitro toxicity assay). In this context, cellular toxicity can be measured by direct cell counts, trypan blue exclusion, or various metabolic activity studies such as 3H-thymidine incorporation, as known to those skilled in the art.

EXAMPLE I

PROTEASE INHIBITION AND DETERMINATION OF ANTIPROTEOLYTIC ACTIVITY IN TEAR SAMPLES

The present example describes representative protocols for determining inhibition of proteolytic activity in. . . rabbits and human subjects treated using the compositions and methods of the invention. This example involves ocular administration of one or more protease inhibitors to suitable test and control subjects, followed by immunological and/or enzymatic assays to identify and quantify resultant proteolytic activity and relevant. . . the following protocols, which exemplify suitable assays for determining caseinolytic activity, are adaptable for additional proteolysis assays using various sample types, target proteases, and protease inhibitor types, concentrations, and formulations (e.g., eyedrops, topical gels, microparticulate solutions, etc.). In particular, the methods described herein below are readily adapted to further define preferred compositions and methods of the invention by determining, e.g., (1) optimal timing and duration of protease inhibitor treatments; (2) optimal inhibitor type(s) for inhibiting different target protease(s) (e.g., by selecting different classes or species of preferred inhibitor(s), or by using inhibitor variants such as recombinant or synthetically modified inhibitors);. . .

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in the following examples are conducted using samples taken from test and control subjects, typically before, during, and after treatment with a protease inhibitor formulation. In general, these assays will incorporate standard proteolytic activity assays along with control assays that determine relevant associated parameters, for example, levels of target proteases and their substrate proteins, quantitative measurements of proteolytic activity (e.g., based on detection of substrate

3 1

protein levels, inhibitor cleavage products, protease /inhibitor complexes, or based on enzymatic assay readings), and levels of endogenous and exogenous protease inhibitors.

Concentration of protease inhibitors and other agents incorporated within
the formulations of the invention will vary in accordance with such factors as the delivery
vehicle. . .

. . .

(pre-conditioned with HPLC buffer) for further analysis. To confirm the relative size range of the eluted material, the column is calibrated, using
protease and antiprotease standards and a commercial mixture of gel filtration
chromatography standards (Bio-Rad).

. . .

Eye. Res. 16:810-819, 1997, incorporated herein by reference). The methods set forth below are described for caseinolytic assays detecting
and/or quantifying selected proteases (elastase, cathepsin G and proteinase-3). protease
33
inhibitors (alp 1, a 1 -Achy, SLP 1, elafin, and a2-M), and protease/inhibitor complexes.

However, these methods are also routinely adaptable for detecting and measuring other
useful proteases and protease inhibitors within the invention, e.g., by substituting the
specific immunological probes, enzymatic reagents, etc.

. . .

per
gel, until the lowest molecular weight marker (See Blue TM markers, Novex, San Diego,
CA) reaches the bottom of the gel. Protease standards, for example human glu-plasmin
(American Diagnostics Inc., Greenwich, CT), cathepsin G, elastase (Calbiochem, La
Jolla, CA) and proteinase-3 (Elastin product Company,. . .

. . .

are selected
to overcome these problems so as to provide useful quantitative data, as demonstrated in
the above cited reports for the protease inhibitors a2-M, alpl (5 8 kDa), a 1 -Achy (6 8 kDa),
SLPI (I 1.7 kDa), elafin (6-9 kDa), cystatin C. . . .

. . .

IgG1
(Jackson Immunochemicals, West Grove, PA and Sigma, St. Louis, MO), which are run,
processed and analyzed simultaneously. Exemplary positive controls for protease
inhibitors include elafin (Peptides International, Louisville, KY), SLPI (R and D System
Inc., Minneapolis, MN), a I -Achy, alpl, a2-M and cystatin. . . and freshly
generated complexes of elastase with alp I and SLPI prepared as detailed by others (Rao
et al., Am. J. Resp. Cell Mol. Biol. 8:612-616, 1993; Stockley et al., Clin. Sci. 66:217-
224, 1984, each incorporated herein by reference).

. . .

detection of reactive products and complexes, anti-bodies with known narrow target antigens are avoided. In this context, exemplary primary antibodies for
detecting proteases and protease inhibitors are
sheep polyclonal antibodies to elastase, a2-

M and alpl (Binding Site), rabbit polyclonal antibodies to cystatin C
(DAKO, Glostrup,
DK), . . .

To estimate concentrations of inhibitor-protease complexes and
degradative products, all detected species are assumed to exhibit
antigenicity similar to
the native antiprotease. Although it is recognized that. . .

Conventional Western blot procedures are too insensitive to allow
detection of nanogram quantities of polymorphonuclear leucocyte (PMN)
cell proteases.

Accordingly, an alternate blotting protocol is employed involving
pre-incubation of the
samples with detergent and a protease inhibitor,
electrophoresis at 4'C (to reduce
adherence and proteolysis), and blot transfer under basic conditions (to
enhance the
36
efficiency of transfer of. . .

Immunoprecipitation Reactions
C tears and the HPLC fractions are immunoprecipitated with antibodies to
a selected protein, glycoprotein, protease or antiprotease by
incubation at VC overnight.

.
shown that
the antiproteolytic compositions and methods of the invention
effectively inhibit
proteolytic activity in extracomeal fluid (tears). By selecting a
multispecific protease
inhibitor (i.e., an inhibitor which targets multiple protease
species) such as a2-M and
alp 1, or by combining multiple protease inhibitors in a
single treatment formulation,
effective inhibition can be achieved against multiple proteases
involved in a corneal
disease process. Accordingly, the methods of the invention will be shown
to inhibit
corneal disease mediated by exposure of the cornea to aberrantly
elevated protease
activities in tears.

Adaptation of the foregoing procedures as disclosed herein will further
demonstrate that antiproteolytic effects against a broad range of
proteases (e.g., acid
esterases, acid phosphatases, acid lipases, cathepsins, collagenases,
elastases, tryptases,
chyrnases, kinins, kalikreins, tumor necrosis factors, chymotrypsins,
stromelysins, and
matrix metalloproteases) that alleviate or prevent corneal disease can
be achieved by
ocular administration of protease inhibitors selected from a
broad range of inhibitors,
including aspartic, serine, cysteine, or metallo-protease
inhibitors. These may be
obtained from natural or artificial sources, and can include modified
inhibitors that vary
in structure from native inhibitors. . .

Species of protease inhibitors useful within this context
include alp 1, a2-M, SLP 1, 0 1 -
37)
antigellagenase, a2-antiplasmin, serine amylyoid A protein, a. . .

EXAMPLE 11

ENHANCED PROTEASE INHIBITION IN CLOSED-EYE TEARS AND ASSOCIATED INHIBITION OF PROTEOLYSIS AND ASSOCIATED HISTOPATHOLOGIC CHANGES IN KERATOCONIC CORNEAS

The present example describes representative protocols for. . . in the cornea of patients suffering from keratoconus. As in the preceding example, this protocol involves ocular administration of one or more protease inhibitors to suitable test and control subjects, followed by immunological and/or enzymatic assays to identify and quantify resultant proteolytic activity and associated. . .

. . . and/or histopathological analyses of corneal tissues, in addition to the above described assays using tear samples. These additional assays allow confirmation that protease inhibition in closed eye tears results in associated inhibition of proteolytic processes in corneal tissues (e.g., as demonstrated by reduction in the activity of specific target protease(s), or by long-term inhibition of histopathological changes such as fragmentation of Bowman's layer).

. . . by reference. Based upon the calculated molar ratios of the observed serpins and the unique presence of two rather than one serine protease binding sites on SLPI, Sathe and coworkers report that SLPI accounts for 95% or more of the total elastase inhibitory activity in. . .

. . . elastase and cathepsin G (Nadziejko et al., Am. J. Resj2. Crit. Care Med. 152:1592-1598, 1995; Rao et al., Am. J. Resp. Cell Mol. Biol. 8:612-616, 1993; Thompson et al., Proc. Natl. Acad. Sci. USA 83:6692-6696, 1986, each incorporated herein by reference), it has no. . . suggests that SLPI and alp I function in a synergistic manner in other mucosal tissue to neutralize the entire spectrum

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of PMN cell serine proteases (Bergenfeldt et al., Scandinavian Journal of Clinical & Laboratogy Investigatio 52:823-829, 1992, incorporated herein by reference).

. . . Miller et al., J. Bacteriol. 171:2166-2172, 1989, each incorporated herein by reference) and inhibits the transmission and replication of HIV virus in cell culture (McNeely et al., J. Clin. Invest. 96:456-464, 1995, incorporated herein by reference). Moreover, in respiratory secretions, its highly basic nature has. . .

24:303-311, 1992; Van-Seuningen et al., Biochem. J. 281:761-766, 1992; Nadziejko et al., Am. J. Respir. Cell & Mol. Biol. 1 1: 103-107, 1994, each incorporated herein by reference). In the respiratory tract, SLPI presumably is localized as. . .

While the serine protease neutralizing capacity of R fluid is limited, in the open eye during inflammation or infection this capacity can be readily augmented through:. . .

Ophthalmol. Vis. Sci. 33:626-640, 1992, incorporated herein by reference). This greatly restricts the capacity of the external ocular environment to neutralize protease activity by passive dilution. Moreover, overnight eye closure is associated with the induction of a subclinical state of inflammation, as evidenced by. . .

et al., Curr. Eye Res. 16:810-819, 1997, each incorporated herein by reference). This results in a stagnant layer enriched in serine proteases and complement products.

The enrichment of serine proteases in closed eye tears raises the question as to how autolytic cell damage is prevented during this period. In further studies, it has been reported that PN4N cell-induced proteolytic damage is avoided at least in part by a build up of four rapid-reacting serpins, with SLPI, alp I and. . . acting as the principal functional entities. These serpins have a combined spectrum of activity such that all known PNIN serine proteases can be inactivated. Sathe et al., Current Eye Res.

However, the majority of a2-M in C tear fluid from most donors remains intact. Notably, although a2-M reacts with PMN cell proteases, it does so at only a relatively slow rate. Swenson et al., J. Biol.

the period of eye closure, there is a sufficient pool of rapid reacting serpins available so as to quench the released proteases in sufficient time to prevent reaction with a2-M. Sathe et al., Current Eye Res. 348-362, 1998, incorporated herein by reference.

Although the closed eye environment is thus uniquely equipped with protective mechanisms against pathogenic infection, including a battery of fast-acting protease inhibitors, it is nonetheless found that the compositions and methods of the invention are particularly useful in preventing proteolytic. . .

Protease Extraction From Corneal Tissues

Prior to extraction, corneal tissues are stored in liquid nitrogen. Tissues are then pulverized in liquid nitrogen using. . .

Eparnatic Assgys

Proteases present in soluble protein preparations from corneal tissues of treated and control subjects are analyzed and quantified by assaying against nitrophenyl acetate as a substrate and by substrate (e.g., gelatin) eletrophoresis.

Smith et al., Eye
9:429-433, 1995, incorporated herein by reference. All proteases
possess acyl transferase
activity and will catalyze the liberation of 4-nitrophenol from
4-nitrophenyl acetate.

. . .
corneal tissues are separated and
visualized after electrophoresis on polyacrylamide gels (8.5%)
containing gelatin (I
mg/ml), as described by Unemori and Werb (L.Cell Biol.
103:1021-31, 1986,
incorporated herein by reference). The sample solutions contain the
ionic detergent
43
sodium dodecyl sulfate (SDS, I% w/v) and glycerol. . . .

To detect levels of exemplary protease inhibitors, the
rehydrated sections are incubated
first in IO% normal goat blocking serum for 20 minutes and then with
polyclonal rabbit
antiliuman. . . .

Additional correlative immunohistochemical assays are conducted to
determine levels of proteases in the corneal samples. For
example, sections are incubated
first in IO% healthy goat or healthy rabbit blocking serum for 3. . .
. . .
scanning microspectrophotometer with the
wavelength set at 5 00 ± IO nni. For each layer of the corneal
specimens, measurements
on five cells from different fields are made. These
measurements are highly reproducible.

Exemplary indicia in this context include quantitative changes in the
extent of
fragmentation of Bowman's layer, fragmentation of the epithelial
cell basement
49
membrane, and/or fibrillation of the anterior corneal stroma. These
indicia can be readily
identified using the immunohistochemical methods set forth above,. . .
. . .
with substantial reduction of specific
proteolytic activity within targeted corneal tissues, which effects are
in turn attributable to
the activity of exogenous protease inhibitors administered
according to the methods of the
invention. Within these methods, preferred embodiments involve
administration of the
inhibitor(s) to a subject. . . .

EXAMPLE III
FORMULATIONS FOR TOPICAL OCULAR DELIVERY OF PROTEASE
INHIBITORS
A variety of topical antiprotease formulations are useful within the
methods of the invention, including solutions, gels, creams, particulate
suspensions, and
the like. For example, eyedrops comprising an aqueous protease
inhibitor solution
isotonic with tears are particularly useful for repeated instillation
during periods of reflex
tear production. However, poor bioavailability of drugs. . .
attributable to a
variety of factors, including rapid clearance of therapeutic agents by
tear flow,

neutralization or dégradation of therapeutic agents by proteases and other mechanisms,
barriers to diffusion and penetration of therapeutic agents across the corneal epithelium to
50
reach the corneal stroma and other. . .

i) Mucoadhesive Polymer Formulations
To address the foregoing delivery issues, the invention provides protease inhibitor compositions wherein the protease inhibitor is dissolved, suspended, or admixed with a delivery vehicle or carrier comprising an opthalmically acceptable, mucoadhesive polymer. Mucoadhesive polymers that adhere. . . each incorporated herein by reference. As used within the compositions and methods of the invention, mucoadhesive carriers prolong contact between the subject protease inhibitor(s) and the underlying absorptive tissue. At the same time, the mucoadhesive carrier ftinctions integrates with, or replaces, naturally adhering mucin until the latter is replenished, which intensified contact weakens barrier properties of the epithelium and results in facilitated penetration of the protease inhibitor(s). These properties of mucoadhesive polymer delivery systems within the invention can be readily determined and optimized in accordance with the following. . . protocols all specifically demonstrate that mucoadhesive polymers, e.g., polycarbophil (a derivative of polyacrylic acid loosely cross-linked with divinylglycol) improves ocular delivery of protease inhibitors in an accepted model system for corneal delivery using the pigmented rabbit.

. . . be obtained from a variety of commercial sources. For the present example, Polycarbophil (Noveon AA-1) is obtained from BF Goodrich (Cleveland, OH). Protease inhibitors are obtained from various sources, as exemplified above. Protease inhibitor levels are measured by any of the foregoing methods or, alternatively by fluorescence polarization immunoassay using a TDx system (Abbott Laboratories,. . .

51
Preparation of Test Formulations
Multiple test formulations are prepared by mixing a selected protease inhibitor with various vehicles, including (1) a 0. 8 1 % (wt/vol) NaCl solution; (2) 0.8 1. % (wt/vol) NaCl and 4.5%. . .

Any one or more of the above enumerated protease inhibitors may be included in the mucoadhesive formulation. In the present example, a2-M and alp I are combined in a series of. . .

Release of Protease Inhibitor In Vitro
Fifty-microliter aliquots from each formulation are added to 5 ml of glutathione-bicarbonate Ringer's solution of pH 7.4 in a. . . for 2

minutes to sediment the polymer particles. A 50]tl sample is withdrawn from the supernatant and assayed to determine concentrations of protease inhibitor(s) after making a 20-fold dilution with TDx-buffer.

to affect the extent of ocular absorption of topically applied pilocarpine, epinephrine, and

52

chloramphenicol. Even if this were not the case for protease inhibitors, comparative evaluations of different delivery formulations should not affected.

Rabbits are kept upright in minimally restraining cases throughout the experiment. The protease inhibitor formulations (25]tl) are instilled using an Eppendorf pipette (Brinkman Instruments, New York, NY) at the upper lid of each. . .

To determine the role of the corneal epithelium in corneal penetration of protease inhibitors, the corneal epithelium is removed before administration of the mucoadhesive formulation by carefully scraping with a lancet blade under topical anesthesia. . .

Aqueous humor and vitreous humor samples are assayed directly utilizing appropriate immunological assays, whereas corneal tissues are subjected to initial processing and protease extraction, as described above.

In accordance with the above methods, the invention provides novel delivery systems comprising a protease inhibitor dissolved or suspended in a mucoadhesive polymer. These compositions are readily optimized in terms of consistency and inhibitor concentration so as to maximally prolong ocular residence time and enhance transport of protease inhibitors and coordinately administered agents to intraocular target sites (e.g., corneal epithelia, stroma, Bowman's layer and vitreous humor compartments). In addition, the. . .

53

ii) Microparticulate Delively Formulations

Although mucoadhesive polymer formulations provide useful vehicles for prolonged ocular delivery of protease inhibitors, microparticulate technology is also shown to provide preferred, long-term ophthalmic delivery vehicles for use within the invention. Methods of the invention. . .

are small polymeric particles (erodible, non-erodible or ion exchange resins) suspended in a liquid carrier medium. These particles contain one or more protease inhibitors in an antiproteolytic effective concentration. Upon administration of the microparticulate suspension in the eye, the particles reside at the delivery site (cul-de-sac, sub conjunctiva or vitreous cavity) and the protease inhibitor(s) is released from the particles

through diffusion, chemical reaction, polymer degradation, or ion-exchange mechanism.

. . .

can also be employed for treating corneal disease according to the methods of the invention, whereby release of effective levels of protease inhibitors can be sustained for long-term periods of 2-3 days and up to two weeks or more. Both corneal and non-corneal. . .

Two general microparticulate forms employed for ophthalmic delivery of antiproteases are microcapsules and microspheres. Particles or aqueous droplets of proteases are entrapped inside a polymeric membrane of microcapsules, yielding almost spherical entities on the order of several hundred microns in diameter. Microspheres are polymeric combinations where the protease inhibitor is homogeneously dispersed in the polymer matrix. Nanoparticles possess similar characteristics of microspheres except that their size is approximately three orders. . .

Time Constant for Chemical Reaction

Release of protease inhibitors through bioerodible polymers is a function of the rate of hydrolysis of the acid or base labile linkages of the polymer and the subsequent release of the protease inhibitor via diffusion through the pores of the matrix created by the polymer hydrolysis. As expected, the time constant for chemical. . .

Microparticulate systems rely on release of protease inhibitors and other therapeutic agents due to diffusion, reaction, physical erosion and ion exchange at the delivery. Each physical or chemical event. . .

. . .

may stimulate reflex tear rate varying between 3 RI to 400 RI per minute. Blinking of the eyelids facilitates spreading of the protease inhibitor in the lacrimal pool over the cornea.

. . .

release of the inhibitor that is predicted or extrapolated by in vitro release kinetics experiments. The primary route by which the protease inhibitor reaches the site of action is the cornea (Lee et al., J. Ocular Pharmacol. 2:67-108, 1986, incorporated herein by reference). However,. . .

The following different approaches can be used to formulate protease inhibitors (and other drugs in combinatorial formulations) in microparticulate dosage form for topical administration.

Polymer-Inhibitor CoMplex

Polymer-inhibitor complexation involves covalent or chemical linking of the protease inhibitor(s) to the polymer backbone forming a macromolecule pro drug.

. . ..

in the polymer matrix. As such, the loaded microparticles are then suspended in a liquid carrier medium, which may also include the protease inhibitor.

These polymers are suitable for release of proteins of different sizes, including full length protease inhibitors and active polypeptide fragments of protease inhibitors.

. . .

acid dimer and sebacic acid of varying molecular weights may be copolymerized for microsphere preparation. The in vitro release of I 0 protease inhibitors from microspheres depends upon the particle size, cross-linking density and protease inhibitor loading. Within the formulations of the invention, near constant or zero order release rates for one week or more can. . .

. . .

acid (L and D form) and liquid crystalline polymer (polyoxyethyelene glycerol tristearate) in microsphere dosage form may also enhance ocular delivery of protease inhibitors. These compositions have inherent ability to modulate inhibitor delivery/release rates in response to small temperature changes in the envirom-nent. Thermoresponsive microspheres. . .

. . .

systems are comprised of antiprotease impregnated nanoparticles suspended in a carrier medium and ready to be directly administered into the eye. The protease inhibitor can be either chemically bound to the polymer backbone or entrapped in the polymer matrix. The preparation of latex particles. . .

Ion exchange delivery systems provide a suitable alternative to circumvent the limitations of diffusional dependent antiprotease release. The availability of free protease inhibitor for diffusion is limited by the exchange kinetics between the inhibitor and the ion exchange resin and the adsorption isotherm.. . .

. . .

is circumvented by fabricating microparticles with molten method technique. However, this technique is plagued by the stability issue relating to both the protease inhibitor and the polymer. The inhibitor is incorporated in the polymer by preparing polymer melt at as high as 180'C. Exposure. . .

. . .

the foregoing procedures and principles, it can be readily demonstrated that a macromolecular pro drug delivery system provides for prolonged delivery of protease inhibitors, while achieving lower peak inhibitor concentration,and consequently resulting in reduced systemic side effects and higher bioavailability. These delivery systems are useful for sustained ocular delivery of protease inhibitors (e.g., 4-8 hr, preferably 8-12 hour, more preferably 12-24 hr) and are also adaptable for long term delivery (e.g., multiple days and up to one to two weeks). Notably, these delivery

vehicles can be readily adapted for optimal delivery of protease inhibitors to the eye to treat corneal disease during periods of closed eye tear production.

EXAMPLE IV
USE OF PRESERVATIVES WITH TOPICAL ANTIPROTEASE FORMULATIONS: PERMEABILITY AND TOXICITY

Deep corneal and intraocular delivery of protease inhibitors is complicated somewhat by the large size of these proteins. Whereas active peptide fragments of protease inhibitors may be efficiently delivered to intraocular compartments, it is more
61
difficult to achieve the same level of penetration with full. . .
. . . .
be particularly useful within the invention include multifaiictional penneabilizing agents. As used herein, multifunctional perineabilizing agents enhanced corneal and intraocular delivery of protease inhibitors and also provide a second, preservative or antibacterial ftmction to the antiprotease composition. In this regard, the present example. . .

In order to evaluate the usefulness of antiprotease formulations containing ophthalmic preservatives, concentrations of the protease inhibitor in tears and corneal tissues are determined for formulations with and without a candidate preservative, before and after instillation of the. . .

Materials Protease inhibitors, e.g., a2-M and alpl, are provided as set forth above. Saponin is obtained from E. Merck (Darmstadt, Germany). Sorbic acid (SA),. . .

Concentrations of protease inhibitors in the tears and corneal tissues is determined by enzyme and/or immunological assays, as above.

CLMEN. . . in a mammalian subject comprising administering to an ocular fluid, surface, or tissue of the subject an antiproteolytic effective amount of a protease inhibitor in an opthalmically acceptable carrier.

2 The method of claim 1, wherein the protease inhibitor is selected from an aspartic, serine, cysteine, or metallo-protease inhibitor.

3 The method of claim 1, wherein the protease inhibitor is recombinantly or synthetically modified.

4 The method of claim 1, wherein the protease inhibitor is administered topically.

6 The method of claim 1, wherein the protease inhibitor is administered during a period of closed eye tear production by the subject.

7. A device for corneal delivery. . . sized and dimensioned to

conform to an external
surface of a human cornea, said inner surface coated or impregnated with
an protease
inhibitor.

.

sized and dimensioned to conform to an external surface
of a cornea of the subject and coated or impregnated with an
protease inhibitor in contact
with the cornea for an effective treatment period sufficient to achieve
delivery of said
protease inhibitor to an ocular fluid, surface, or tissue of
the subject.

11 A pharmaceutical formulation for treating corneal disease in a
mammalian subject comprising an antiproteolytic effective amount of a
protease inhibitor
in an opthalmically acceptable carrier.

12 The pharmaceutical formulation of claim 1 1, wherein the
protease
inhibitor is selected from an aspartic, serine, cysteine, or metallo-
protease inhibitor.

13 The pharmaceutical formulation of claim 1 1, wherein the
protease
inhibitor is recombinantly or synthetically modified.

14 The pharmaceutical formulation of claim 1 1, wherein the
protease
5 is formulated in a carrier adapted for topical administration selected
from a topical
solution, cream, gel, or microparticulate carrier.

16 The pharmaceutical formulation of claim I 1, comprising a plurality
of protease inhibitors.

17 The pharmaceutical formulation of claim 16, comprising a plurality
of multispecific protease inhibitors.

18 The pharmaceutical formulation of claim 17, wherein at least one
of said protease inhibitors is selected from a2-M, alp 1, or
SLP 1.

19 The pharmaceutical formulation of claim 16, comprising a first,
multispecific protease inhibitor and a second, oligospecific
or specific protease inhibitor.
66
. The pharmaceutical formulation of claim 19, wherein at least one
of said protease inhibitors is selected from a2-M, alpl, SLPI,
PI-antigellagenase, a2-
antiplasmin, serine amylyoid A protein, al-antichymotrypsin (al-Achy),
cystatin C,
inter-a-trypsin inhibitor, elafin, elastinal,. . .

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'IS' IS NOT A VALID FORMAT FOR FILE 'PCTFULL'

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ALLG------------ALL, MAX plus GI
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IND, IPC--------ICM, ICS
ABS--------------ABEN, ABF, ABFR, ABDE, ABES
TX--------------DETD, CLM
IALL,IMAX-------ALL indented with text labels
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     (FILE 'HOME' ENTERED AT 13:58:41 ON 20 SEP 2006)

     FILE 'PCTFULL' ENTERED AT 13:58:51 ON 20 SEP 2006
L1             1 S WO2001030380/PN
L2             0 S L1 AND ANTIPROTEINASE
L3             0 S L1 AND ?ANTIPROTEINASE
L4             1 S L1 AND PROTEASE
L5             1 S L4 AND ANTI?
L6             1 S L4 AND CELL

=> s l1 and contact? or administ?
        432156 CONTACT?
        167483 ADMINIST?
L7      167483 L1 AND CONTACT? OR ADMINIST?

=> s l1 and (contact? or administ?)
        432156 CONTACT?
        167483 ADMINIST?
L8           1 L1 AND (CONTACT? OR ADMINIST?)

=> d kwic

L8      ANSWER 1 OF 1      PCTFULL   COPYRIGHT 2006 Univentio on STN
PI      WO 2001030380         A2 20010503
ABEN    Compositions and methods for treating corneal diseases mediated by
        elevated protease activity include ocular administration of
        protease inhibitors. One or more protease inhibitors selected from an
        aspartic, serine, cysteine, or metallo-protease inhibitor are
        administered to an ocular fluid, surface, or tissue, preferably
        by topical administration, to inhibit proteolytic activity
        associated with a corneal disease or condition, for example keratoconus.
        Antiproteolytic formulations of the invention may.   .   . also include
        other therapeutic agents such as antiinflammatory or antibiotic drugs.
        In preferred aspects of the invention, antiproteolytic formulations are
        administered during periods of closed eye tear production. Also
        provided within the invention are implant devices for corneal delivery
        of a.   .   .

DETD    .   .   .  have been developed to treat or prevent
        keratoconus. In the mildest cases, management involves the use of
        spectacles or soft
        1 5 contact lenses. More commonly, early stage management of
        keratoconus requires
```

specially designed contact lenses that compensate visual defects and provide some structural support to correct corneal distortion. More advanced presentations are managed with rigid gas-permeable (RGP) contact lenses to minimize corneal distortion and correct irregular astigmatism. Koliopoulos et al., Ann. Oplithalmol. 13(7):835-7, 198 1, incorporated herein by reference. If satisfactory wearing time is not achieved with contact lens, or if the contact lens-corrected vision is not adequate (which may result from corneal scarring or poor fitting of the steeply sloped cone) keratoplasty is. . .

. . .

Int. Ophthalmol. Clin. 33:249-260, 1993, each incorporated herein by reference. Other reports suggest that environmental conditions, such as excessive eye rubbing or contact lens wearing, contribute to the disease. Coyle, Am. J. Ophthalmol.

Other reports in the literature suggest that rigid contact lens wear is a causal factor in some cases of keratoconus. Macsai et al., Arch. Ophthalmol. 108:534-8, 1990, incorporated herein by reference. This finding is difficult to substantiate, because keratoconus patients may self-select contact lens wear due to their refractive error or patient dissatisfaction with spectacles.

. . .

simple genetic models for the disease are complicated by, or conflict with, models that embrace mechanical factors (e.g., eye rubbing or contact lens wear) as causes for the disease. Yet additional environmental causes, including atopic disease and systemic conditions, further complicate etiological modeling of. . .

. . .

It is a further object of the invention to achieve the foregoing objects within methods and compositions that are easy to administer and which employ formulations that optimize delivery of therapeutic agents to ocular target sites including extracorneal fluid (tears, aqueous humor, or vitreous. . .

. . .

atopic disease, and visual impairment. In more detailed aspects, methods and compositions for treating keratoconus are adapted for combinatorial use with corrective contact lenses, including rigid gas-permeable (RGP) lenses.

The methods of the invention involve ocular administration of an antiproteolytic effective amount of a protease inhibitor in an opthalmically acceptable carrier. The protease inhibitor is preferably a protein or. . .

According to the methods of the invention, a protease inhibitor formulation is administered to an ocular fluid, surface, or tissue, preferably by topical administration, in an antiproteolytic effective amount to substantially inhibit a proteolytic activity associated with the corneal disease or condition to be

treated.. . .

. . .

of the
invention vary in accordance with a variety of factors detailed below.
In preferred
aspects of the invention, antiproteolytic formulations are
administered during periods
when contact lens are wom and/or of closed eye tear production
to enhance therapeutic
efficacy.

. . .

the
17
form of an ocular implant having a concave inner surface similar in size
and shape to the
inner surface of a contact lens. The device is applied
externally to the cornea of a patient
suffering from a corneal disease or condition and serves. . .

. . .

provides useful methods and compositions for
treating or preventing corneal disease in a mammalian patient. The
methods of the
invention involve ocular administration of an antiproteolytic
effective amount of a
I 0 protease inhibitor in an opthalmically acceptable carrier. The
protease inhibitor is
preferably selected. . .

According to the methods of the invention, a protease inhibitor
formulation is administered to an ocular fluid, surface, or
tissue, preferably by topical
administration, in an antiproteolytic amount effective to
substantially inhibit a proteolytic
activity associated with the corneal disease or condition to be treated.

By substantial inhibition of proteolytic activity is meant that
administration of the protease inhibitor formulation yields at
least about a IO% reduction
of proteolytic activity, preferably at least a 20% reduction,. . . at
an ocular target site, for example within the extracomeal fluid,
corneal epithelium, corneal stroma, Bowman's layer, or the vitreous
humor. Preferably,
administration of the protease inhibitor yields approximately
a 30-50% reduction of
proteolytic activity, more preferably greater than about a 50%
reduction, and. . .

. . .

to be treated, for example keratoconus or
corneal infections. As further defined herein, target proteases are
amenable to regulatory
inhibition by exogenously administered protease inhibitors.

. . .

may
include extracorneal fluid or corneal tissue samples taken from subjects
(e.g., a human or
non-human mammal such as a rabbit) following administration of
a protease inhibitor
formulation (test sample), and following administration of a
placebo comprising, e.g., a
selected carrier without the protease inhibitor (control sample). Often,
test and control
samples will be provided by bilateral administration of test
and control treatments to an
individual patient. Other suitable test and control samples will be
determined by those

skilled in. . .

For practicing the methods of the invention, the precise amounts of protease inhibitors to be administered and the frequency and duration of treatment will depend on the status of the corneal condition or disease to be treated, and on other factors such as the patient's state of health and weight, the mode of administration, the nature of the formulation, etc. These factors will vary such that specific regimens can be established by those skilled in the art to maximize efficacy of treatment. Ordinarily, the antiprotease is administered in a dosage of between approximately 0.2]tg/ml and 1.0
24
mg/ml. Preferably,theinhibitorispresentinaconcentrationofaboutO I.Ogg/ml, more preferably at a concentration of about 0.5 gg/ml. Exemplary. . . the inhibitor at approximately the same range of concentrations, with the most preferred concentration being between about 1.0 and 5.0 gg/ml. The administration schedule can range from a continuous infusion, to once or twice a day, up to 6 or more administrations a day, with dose levels and administration protocols being selected by the health professional. Administration onto the concave surface of contact lenses before insertion into the eye is an effective method of enhancing the residence time for the solution in contact with the comea.

. . . .
the form of a one time dose, e.g., in the context of sustained delivery and long-term delivery formulations described below. Alternatively, multiple administrations may be indicated and, under certain circumstances, continuous treatments may be selected.

In a preferred aspect of the invention, compositions comprising a protease inhibitor are administered during periods of closed eye tear production (e.g., during a patient's sleep periods). This method greatly enhances antiproteolytic results, yielding prolonged inhibition. . . demonstrated by reduction in the activity of specific target protease(s)), and long-term inhibition of histopathological changes, such as fragmentation of Bowman's layer. Administration of the antiprotease compositions of the invention during periods of closed eye tear production greatly enhances the antiproteolytic efficacy of these compositions compared to the efficacy achieved by antiprotease administration during periods of reflex tear production, although the latter use is effective and within the scope of the invention. This is due. . . that characterize the closed eye, versus reflex tear environments. The protease inhibitor compositions and pharmaceutical formulations of the invention can also be administered during a period that is concurrent with or closely preceding a medical procedure or other event anticipated to produce a risk of proteolytic

injury, for example following eye surgery or during bacterial infection. Thus, methods are provided which involve administration of an antiproteolytic composition concurrent with, or within an antiproteolytic effective period preceding or following, a surgical procedure or infection, whereby the administration reduces or eliminates risk of deleterious proteolytic responses normally associated with the procedure or infection.

. mixture of one or more protease inhibitors combined with a second therapeutic agent (e.g., an antibiotic, antiviral or antiinflammatory drug) can be administered by a variety of routes, including via topical administration (using, e.g., drops, gels, creams or microparticles as carriers), injection (e.g., via hypodermic or pneumatic introduction into the cornea or vitreous humor).

Preferred methods of the invention involve coordinate (e.g., simultaneous or closely contemporaneous to yield coordinate treatment) administration of a plurality of antiprotease proteins, analogs, salts, or derivatives, or administration of formulations comprising multiple protease inhibitors that may be admixed or complexed. Practice of these methods reduces abnormal proteolytic mechanisms attending a targeted corneal disorder (e.g., keratoconus) at combinatorial antiproteolytic levels that exceed antiproteolytic levels observed when either of the coordinately administered protease inhibitors are administered alone. This inhibition, as when other compositions and methods of the invention are employed, reduces proteolytic activity in extracomeal fluid (tears), and. . . a multispecific protease inhibitor (i.e., an inhibitor which targets multiple protease species), such as a2-M, SLP I and alp 1, is coordinately administered with another multispecific inhibitor, or, alternatively, a multispecific inhibitor is coordinately administered with an oligospecific or specific inhibitor (the latter types of inhibitors represented, e.g., by 0 1 -antigellagenase, a2-antiplasmin, serine amylyoid A protein,. . .

Additional preferred methods of the invention involve coordinate administration of an antiprotease and an antibiotic, or administration of fon-nulations comprising both a protease inhibitor and an antibiotic. Practice of these methods reduces abnormal proteolytic mechanisms attending a targeted corneal. . .

Typically, the protease inhibitors, analogs, salts, derivatives, and coordinately administered therapeutic agents of the invention will be administered in the form of a pharmaceutical composition, i.e., dissolved or suspended in a physiologically

acceptable carrier, preferably an aqueous carrier. A variety. . . art and readily formulated with the subject therapeutic agents, including biologically compatible gels, creams, microparticulate solutions and the like suitable for topical administration. The pharmaceutical compositions may be sterilized by conventional, well known sterilization techniques. The resulting formulations may be packaged for use or filtered under aseptic conditions and lyophilized, the lyophilized preparation being combined with a sterile aqueous solution or other carrier prior to administration.

In other embodiments of the invention, the protease inhibitors, analogs, salts, derivatives, and coordinately administered therapeutic agents of the invention are prepared with carriers that protect the compound against rapid elimination from the ocular environment, such as. . .

. . . .

conforms to an external surface of the cornea, i.e., which is similar in size and shape to the inner surface of a contact lens, and is applied externally to the cornea of a patient suffering from a corneal disease or condition. The device serves. . .

. . . .

or 20% by weight, and will be selected primarily by fluid

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volumes, viscosities, etc., in accordance with the particular mode of administration selected.

In preferred methods within the invention, mammalian subjects, including human patients, suffering from protease-mediated corneal disorders are treated by administering to the patient a pharmaceutical or therapeutic composition comprising an effective amount of one or more antiproteases, or a pharmaceutically acceptable derivative. . .

. . . .

deliver to a patient an antiinflammatory effective amount without causing serious adverse side-effects in the patient treated. The active compound is preferably administered to achieve peak concentration of the antiprotease in tear fluid or corneal tissue of the patient within about 1-4 hours after administration. Concentration of the antiprotease in pharmaceutical compositions and devices of the invention will depend on such factors as absorption, distribution,

1 5. . . understood that for any particular subject, specific dosage regimens may be adjusted over time according to the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope. . .

The pharmaceutical formulations administered within the methods of the

invention must be opthalmically acceptable. In general, compounds and formulations
with a therapeutic index of at least. . .

. . . .

in extracorneal fluid (tears) of rabbits and human
subjects treated using the compositions and methods of the invention. This example
involves ocular administration of one or more protease
inhibitors to suitable test and
control subjects, followed by immunological and/or enzymatic assays to identify and
quantify. . .

. . . .

suitable control subjects, for example control subjects that
present similar pathologies as test subjects but are differentially treated (e.g., using a
placebo administration, altered dose, alternative timing or
mode of administration, etc.),
or control subjects that have healthy corneas and are treated similarly to diseased test
subjects.

. . . .

timing, and duration of treatment is also varied as described in
further detail below. Most commonly, antiproteolytic formulations of the invention are
administered topically, e.g., in the form of drops, gels,
creams, microparticulate solutions,
and the like. Alternatively, antiproteolytic formulations are delivered directly to. . ..

For determining short-term delivery kinetics and efficacy, antiproteolytic
I 0 formulations are administered to subjects in a one-time
dose, and proteolytic activity and
associated parameters are determined for samples taken at selected periodic time
increments (e.g., at I hr, 4 hr, 8 hr, 16 hr, and 24 hr post-administration). For determining
longer-term delivery kinetics and efficacy, antiproteolytic formulations are administered
to subjects in a single, sustained-delivery dose, or in multiple doses (e.g., daily), and
measurements of proteolytic activity and associated parameters are. . .

. . . .

tryptases,
chyrnases, kinins, kalikreins, tumor necrosis factors, chymotrypsins, stromelysins, and
matrix metalloproteases) that alleviate or prevent corneal disease can be achieved by
ocular administration of protease inhibitors selected from a
broad range of inhibitors,
including aspartic, serine, cysteine, or metallo-protease inhibitors. These may be
obtained from. . .

Quantitative analyses based on the foregoing procedures will further
demonstrate that administration of the antiproteolytic
compositions of the invention yields
at least about a 20% reduction of proteolytic activity compared to a relevant baseline or
control value at an ocular target site. Moreover, when these compositions are
administered in preferred formulations and treatment protocols
in accordance with the

examples below, proteolytic inhibition of approximately 50-80% compared to baseline/control values can be achieved, while in some instances administration of these compositions will yield effective neutralization of proteolytic activity corresponding to a reduction of about 85-100% of baseline/control proteolytic activity.

. . .

cornea and associated histopathological changes in the cornea of patients suffering from keratoconus. As in the preceding example, this protocol involves ocular administration of one or more protease inhibitors to suitable test and control subjects, followed by immunological and/or enzymatic assays to identify and. . .

However, the present example is specifically directed to administration of an antiprotease composition to patients during periods of closed eye tear (C tear) production. In addition, the protocol of the instant. . .

38

Administration of the antiprotease compositions of the invention during periods or closed eye tear production greatly enhances the efficacy of corneal therapy according. . .

. . .

that the compositions and methods of the invention are particularly useful in preventing proteolytic damage and attendant corneal pathogenesis when administered during closed eye periods.

For determining and quantifying the effects of antiproteolytic compositions administered during periods of closed tear production, both animal and human subjects may be used in accordance with the preceding example. The. . . above are employed, with the modification that test and control samples are represented by subjects to whom an antiproteolytic composition is administered, at similar doses and for comparable duration, entirely during a reflex tear or closed eye tear production period, respectively. Values obtained from. . . are compared against one another after normalization to baseline values (e.g., values determined in baseline control samples to which a placebo is administered) and determination of the associated parameters outlined above. These results thus enable confirmation that the compositions and methods of the invention yield. . .

In addition to the foregoing analyses, alternate test and control samples are utilized to document that closed eye administration of antiproteolytic compositions of the invention results in quantitative inhibition of proteolysis, not only in the extracorneal fluid, but also in the corneal tissue of treated subjects. For this purpose, the

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antiproteolytic compositions are administered, as above, to rabbit subjects during closed

eye and reflex tear production periods. A protracted treatment regimen is employed, wherein the antiproteolytic composition is administered daily for one week. The subjects are then sacrificed and corneal tissues are isolated and processed for analysis of proteolytic activity. These. . .

Additional assays are also utilized herein to document that administration of antiproteolytic compositions of the invention results in quantitative inhibition of structural degradative changes in the corneas of patients suffering from keratoconus.. . . corneal tissues will be incidentally available for harvest. Prior to the scheduled surgical procedure, antiproteolytic compositions of the invention and placebo formulations are administered to test and normal control groups of patients during closed eye and reflex tear production periods. This treatment protocol involves an even more protracted regimen in order to manifest inhibition or reversal of corneal structural changes, wherein the antiproteolytic composition is administered daily for two to six months prior to surgery. At the time of surgery, corneal tissues are harvested and a portion. . .

. . .

reduction of specific proteolytic activity within targeted corneal tissues, which effects are in turn attributable to the activity of exogenous protease inhibitors administered according to the methods of the invention. Within these methods, preferred embodiments involve administration of the inhibitor(s) to a subject during a period of closed eye tear production.

. . .

Pharin. Res. 8:1039-1043, 1991, each incorporated herein by reference. As used within the compositions and methods of the invention, mucoadhesive carriers prolong contact between the subject protease inhibitor(s) and the underlying absorptive tissue.. At the same time, the mucoadhesive carrier ftinctions integrates with, or replaces, naturally adhering mucin until the latter is replenished, which intensified contact weakens barrier properties of the epithelium and results in facilitated penetration of the protease inhibitor(s). These properties of mucoadhesive polymer delivery. . .

To determine the role of the corneal epithelium in corneal penetration of protease inhibitors, the corneal epithelium is removed before administration of the mucoadhesive formulation by carefully scraping with a lancet blade under topical anesthesia elicited by 25gl of proparacaine (0.025%) and general. . .

. . .

terms of consistency and inhibitor concentration so as to maximally prolong ocular residence time and enhance transport of protease inhibitors and coordinately administered agents to

intraocular target sites (e.g., corneal epithelia, stroma, Bowman's layer and vitreous humor compartments). In addition, the delivery systems are safe. . . .

exchange resins) suspended in a liquid carrier medium. These particles contain one or more protease inhibitors in an antiproteolytic effective concentration. Upon administration of the microparticulate suspension in the eye, the particles reside at the delivery site (cul-de-sac, sub conjunctiva or vitreous cavity) and. . . .

following different approaches can be used to formulate protease inhibitors (and other drugs in combinatorial formulations) in microparticulate dosage form for topical administration. . . .

temperature of the environment. The particle swelling results in increased viscosity of the formulation in situ, thereby prolonging the dosage form cornea contact time. Inhibitor impregnated ultra fine microspheres of methyl methacrylate and acyclic acid polymer with diameters ranging from 0.2 to 1.0 gm. . . .

In-Situ Gelliny, Latex Nanovarticles

These systems are comprised of antiprotease impregnated nanoparticles suspended in a carrier medium and ready to be directly administered into the eye. The protease inhibitor can be either chemically bound to the polymer backbone or entrapped in the polymer matrix. The. . .

The latex suspension, when instilled into the eye, and upon coming in contact with the lacrimal fluid at pH 7.2 to 7.4 gels in situ and is thus resistant to rapid wash out of. . .

Since latex preparations consist of up to 30% w/v of the polymer, the topical ocular administration may result in transient vision blurring, caking of the eyelids and forceful closing of the eye due to the viscid nature. . .

The foregoing protocols allow demonstration that antiprotease fort-nulations containing ophthalmic preservatives delivered via an ocular route increase the penneability of ocularly administered antiproteases. Thus, these formulations are particularly useful within the invention by their ability to simultaneously stabilize fon-nulations, inhibit microbial infection, and enhance. . .

CLMEN I . A method for treating corneal disease in a mammalian subject comprising administering to an ocular fluid, surface, or tissue of the subject an antiproteolytic effective amount of a protease inhibitor in an opthalmically. . .

4 The method of claim 1, wherein the protease inhibitor is administered topically.

6 The method of claim 1, wherein the protease inhibitor is administered during a period of closed eye tear production by the subject.

1 5 7. A device for corneal delivery of an. . . .

```
. . .
      to conform to an external surface
      of a cornea of the subject and coated or impregnated with an protease
      inhibitor in contact
      with the cornea for an effective treatment period sufficient to achieve
      delivery of said
      protease inhibitor to an ocular fluid, surface, or. . .

      14 The pharmaceutical formulation of claim 1 1, wherein the protease
      5 is formulated in a carrier adapted for topical administration
      selected from a topical
      solution, cream, gel, or microparticulate carrier.


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                                                          ENTRY    SESSION
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